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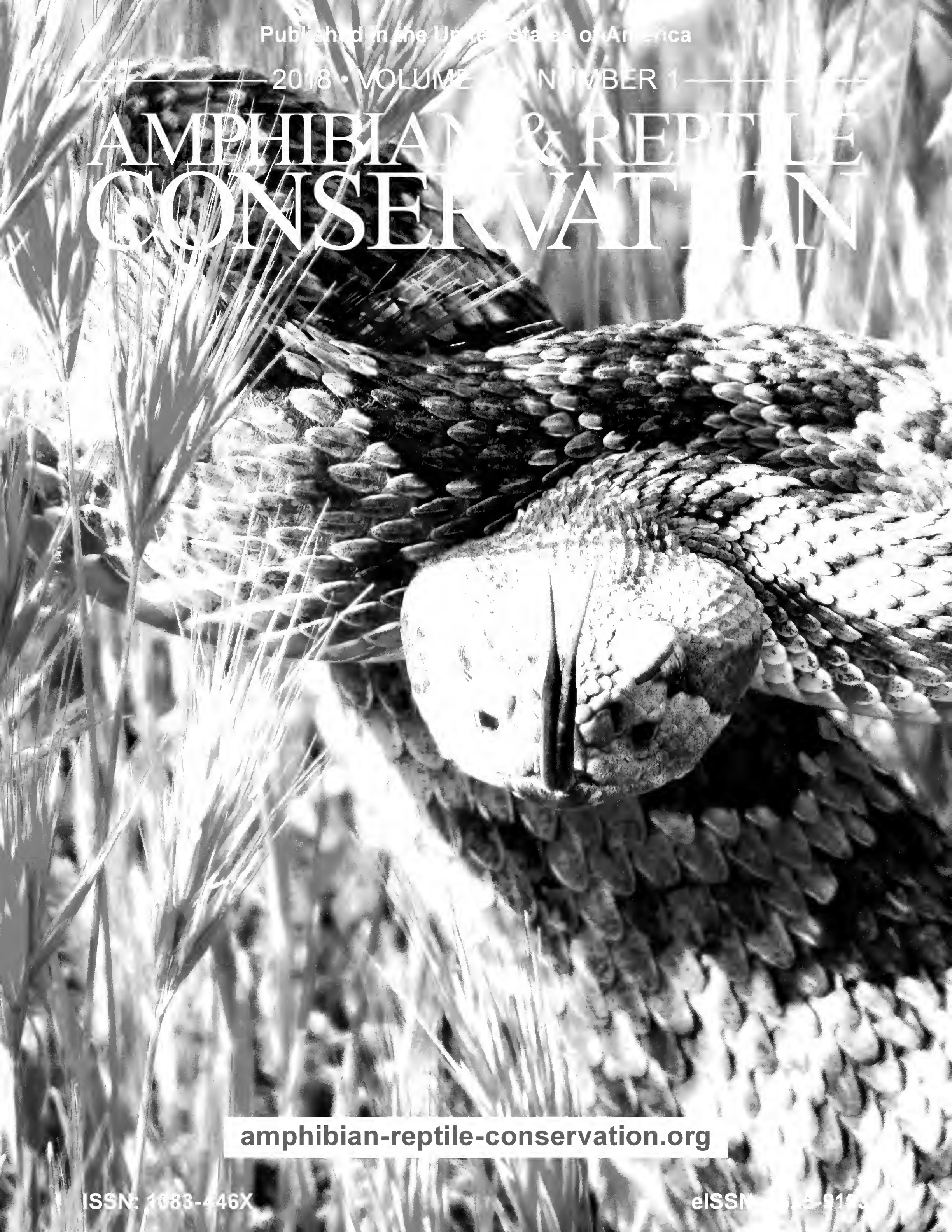

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New distributional records of the Toad-headed Pitviper *Bothrocophias hyoprora* (Amaral, 1935) in Brazil

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Keywords. Viperidae, snake, Amazonian herpetofauna, Canutama, Altamira, distribution extension

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The genus *Bothrocophias* Gutberlet and Campbell 2001 is a monophyletic entity composed of six species: *Bothrocophias andianus* (Amaral, 1923), *B. campbelli* (Freire- Lascano, 1991), *B. colombianus* (Rendahl and Vestergren, 1941), *B. hyoprora* (Amaral, 1935), *B. microphthalmus* (Cope, 1875), and *B. myersi* Gutberlet and Campbell, 2001 (Carrasco et al. 2012). It is widely distributed in tropical lowland forests of the Amazon basin of Colombia, Ecuador, Peru, Bolivia, and Brazil (Campbell and Lamar 2004; Fenwick et al. 2009; Carrasco et al. 2012; Wallach et al. 2014).

Among the *Bothrocophias* species, the Toad-head Pitviper (*B. hyoprora*) exhibits the widest distribution, occurring in lowland Amazonian forests of Colombia, Ecuador, Peru, Bolivia, and Brazil (Campbell and Lamar 2004; Cisneros-Heredia et al. 2006). In the Brazilian Amazon, the species is broadly distributed from the western Amazonas to the eastern middle Tapajós River, also occurring at the states of Acre, Rondônia, and Mato Grosso (Bernarde et al. 2011; Mendes-Pinto and Souza 2011; Carvalho et al. 2013). According to the available literature, *Bothrocophias hyoprora* is often found on the leaf litter near water bodies (Campbell and Lamar 2004), and feeds upon centipedes, anurans, lizards, and rodents (Martins and Oliveira 1998; Martins et al. 2002).

We herein report two vouchered specimens and an additional non-collected specimen of *B. hyoprora* from southwestern Pará and southern Amazonas, which is located in northern Brazil (Fig. 1). An adult male of *B. hyoprora* (MPEG 24662, snout-vent length 366 mm, tail length 82 mm) was collected on 2 April 2011 by L. Drummond, H. Costa, and J. Tonini, in an ombrophilous dense forest located in Jardim do Ouro, eastern part of the Itaituba municipality, state of Pará, Brazil (6.26190°S, 55.90621°W; WGS 84; 237 m). The specimen is deposited in the herpetological collection "Oswaldo Rodrigues da Cunha," Museu Paraense Emílio Goeldi, Belém, Brazil – MPEG. An adult male (INPA-H 33106, snout-vent length 347 mm, tail length 63 mm; Fig. 2) was collected on 24 April 2013 by Alexandre Almeida and F. Assunção, in a dense forest in the Floresta Estadual Canutama, a Conservation Unit on Canutama municipality, southern Amazonas, on the right bank of the Paissé River (6.49514°S, 64.56611°W; WGS 84; 75 m). This specimen is deposited in the herpetological section of the Zoological Collections of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil – INPA-H. An adult *B. hyoprora* (UF 157255; Fig. 3) was found on 18 April 2016 by Ivanei Araújo and Edson Reis in a preserved forest transect in the of the Chapleau mining company, concession (7.550479°S, 55.034344; WGS 84; 238 m), Altamira municipality, Pará state, Brazil. This record corresponds to a photographic voucher specimen deposited at the Florida Museum of Natural History–UF.

The register represented by the MPEG specimen extends the known distribution of *Bothrocophias hyoprora* ca. 190 km south from the last known record, which was at FLONA Trairão, Pará. The University of

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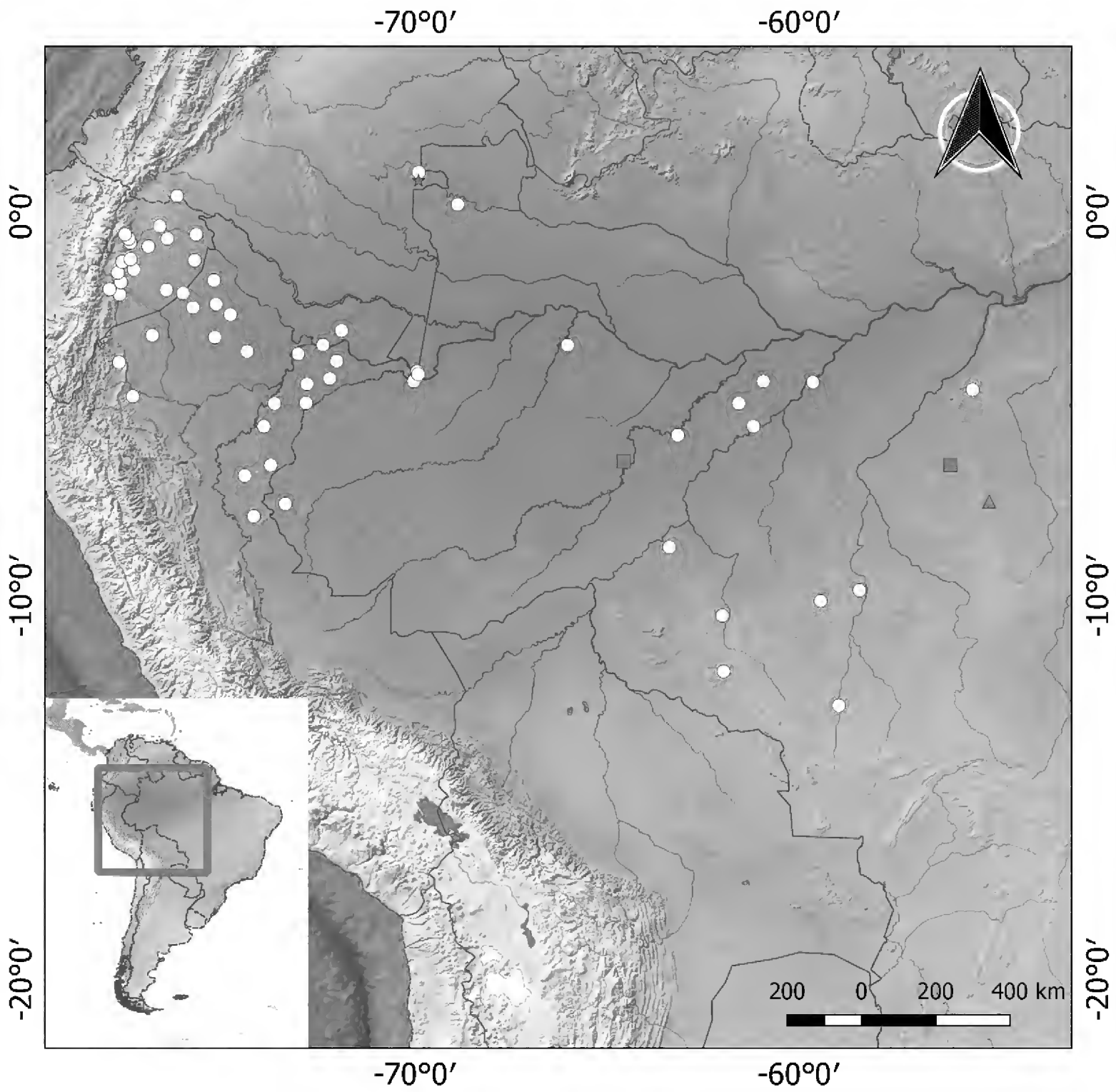

Fig. 1. Known geographic range of *Bothrocophias hyoprora* in South America: white circles = literature data, red star = type locality, red squares = records from Jardim do Ouro, Itaituba, Pará, Brazil (MPEG 24662) and from Floresta Estadual Canutama, Canutama, Amazonas, Brazil (INPA-H 33106), red triangle = record from Chapleau mining company concession, Altamira, Pará, Brazil (UF 157255).

Fig. 2. Adult *Bothrocophias hyoprora* (INPA-H 33106) from Canutama, Amazonas, Brazil. *Photography by Vinícius T. de Carvalho.*

Fig. 3. Adult *Bothrocophias hyoprora* (UF 157255) from Altamira, Pará, Brazil. *Photography by Ivanei S. Araujo.*

Florida photographed specimen expands the distribution ca. 270 km southeast (Mendes-Pinto and Souza 2011). Both records fill a distributional gap in the Xingu-Tapajos interfluve, located in southeast Pará. The INPA specimen fills an important gap on southern Amazonas, at the Juruá-Purus interfluve, one of the most unexplored region of Amazonia concerning the herpetofauna. These records provide new distribution data about this rare species in the Brazilian Amazon. The UF record is the first for the municipality of Altamira. Despite being considered abundant at the Andean slopes of Colombia, Peru, and Ecuador, *Bothrocophias hyoprora* records are very uncommon in Brazil, with few specimens being registered for central and western Amazon. However, the lack of registers is most likely due to scarcity of field work rather than low demographic of the species in the region (Carvalho et al. 2013).

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On the occurrence of *Hemiphractus scutatus* (Spix, 1824) (Anura: Hemiphractidae) in eastern Amazonia

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Abstract.—Hemiphractus **Wagler, 1828 is part of Hemiphractidae Peters, 1862, a family that harbors species of frogs characterized by the deposition of eggs on the females' dorsum. Both the genus *Hemiphractus* and the species *Hemiphractus scutatus* (Spix, 1824) are only known to the Andean mountain range and western half of the upper Amazon Basin. Herein, we provide the first records of *H. scutatus* from the eastern Amazonia (middle Tapajós River region, Pará State, Brazil), which extends its geographic range ca. 1,000 km from nearest known occurrence record and are among the lowest known levels for the species elevational range. Comparisons of morphologic and molecular data with available voucher specimens and published information on the species revealed variation that we interpret as intraspecific polymorphism. Phylogenetic analysis of a fragment of the mitochondrial gene *16S* recovered the newly discovered specimens as most closely related to samples from Peru. These results add new evidence in the known biogeographic patterns of the genus and species, and ongoing plans to build hydroelectric plants in the middle Tapajós River region can negatively affect this unique population.**

Keywords. Biogeography, conservation, geographic range, marsupial frogs, morphology, Pará State, phylogenetic relationships

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Introduction

According to the latest phylogenetic revisions (Castroviejo-Fisher et al. 2015; Duellman 2015), the family Hemiphractidae Peters, 1862 is considered monophyletic and include the genera *Cryptobatrachus* Ruthven, 1916 (six species), *Gastrotheca* Fitzinger, 1843 (70 species), *Stefania* Rivero, 1968 (19 species), *Flectonotus* Miranda-Ribeiro, 1926 (two species), *Fritziana* Mello-Leitão, 1937 (five species), and *Hemiphractus* (six species). Members of this family inhabit humid Neotropical forests in different elevational zones: Central America, Chocó, Andes, mountainous Caribbean coast, the island of Trinidad and Tobago, Amazonia, and the Atlantic Forest (Castroviejo-Fisher et al. 2015; Duellman 2015). These frogs share a unique reproductive mechanism, with deposition of eggs on the females' dorsum (Duellman 2015). In Amazonia, this characteristic seems to be relevant to define hemiphractid geographic ranges, as they are more diverse and abundant in the west, which may be a result of ideal climatic conditions for its life cycle in this region (Bernal and Lynch 2013; Duellman 2015), such as the lower seasonality and higher annual rainfall (Sombroek 2001).

The species of the genus *Hemiphractus* are terrestrial and arboreal frogs with well-modified and ornamented skulls (Trueb 1974), which are distributed throughout Central America, East of Andes and in the extreme western Amazon basin (Frost 2017): *H. bubalus* (Jimenez la Espada, 1870), *H. fasciatus* Peters, 1862, *H. helioi* Sheil, and Mendelson III, 2001, *H. johnsoni* (Noble, 1917), *H. proboscideus* (Jimenez de la Espada, 1870), and *H. scutatus* (Spix, 1824). The latter is the type species of the genus and inhabits a wide elevational range along the western Amazon Basin and Andean mountain range, in Bolivia, Peru, Ecuador, and Brazil (Spix 1824; Myers and Carvalho 1945; Trueb 1974; Duellman and Lynch 1988; Rodríguez and Duellman 1994; Ruiz-Carranza et al. 1996; Sheil and Mendelson III 2001; Lehr 2001; Moravec et al. 2002; Coloma et al. 2004; Duellman 2005;

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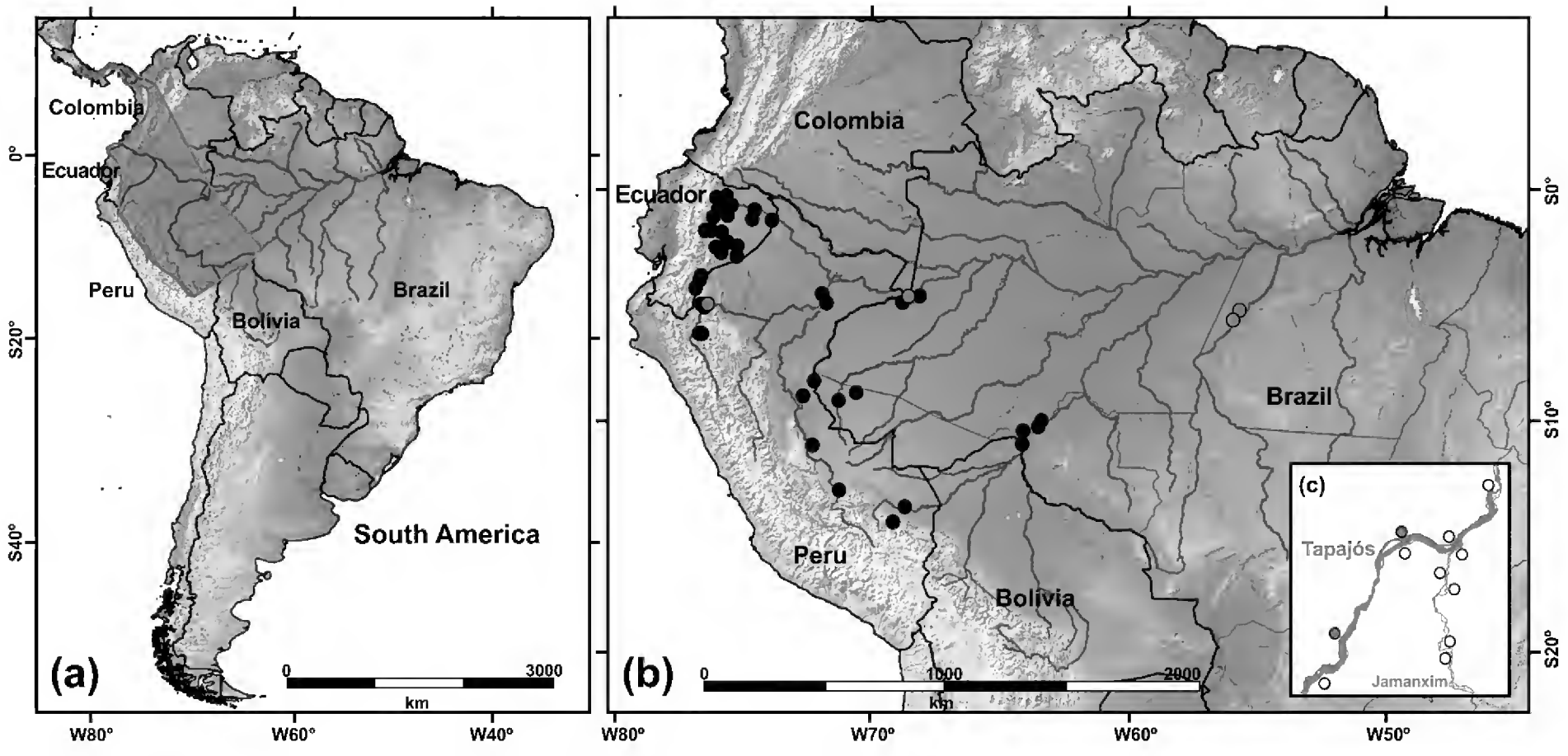

Fig. 1. Known distribution of **(a)** genus *Hemiphractus* (in purple) and **(b)** *Hemiphractus scutatus* (dots), highlighting new locality of occurrence in middle Tapajós River region, Pará State, Brazil (red dots) and localities of sequences included in molecular analysis (yellow dots). The region of new records is zoomed in (c), showing the sampling sites where *H. scutatus* was present (red) and not recorded (white).

Lynch 2005; Cisneros-Heredia 2006; Glaw and Franzen 2006; Muñoz-Saravia 2008; Souza 2009; von May et al. 2009; Beirne and Whitworth 2011; Bernarde et al. 2011; Catenazzi et al. 2013; Ortiz 2013; Almendáriz et al. 2014; Castroviejo-Fisher et al. 2015; Frost 2017; GBIF 2017; Rainforest Conservation Fund 2017; SpeciesLink 2017).

Herein we present the first records of *Hemiphractus scutatus* from the middle Tapajós River region, Pará State, Brazil. These records are the easternmost known localities of occurrence reported for this species and the genus, and are among the lowest known elevational levels for the species distribution. We also present a phylogenetic tree based on mtDNA gene *16S* for some *Hemiphractus* species, and discuss on the biogeographic implications of these records and conservation of this population.

Material and Methods

The amphibian survey was conducted on the middle Tapajós River region, Pará State, Brazil. This river is one of the largest tributaries of the Amazon River (Sioli 1968) and is located in eastern Amazonia. The climate in this region have a high seasonality (Sombroek 2001), with average annual temperature of 26 °C and total annual rainfall exceeding 2,400 mm (Wang et al. 2017), with driest months from June to August (Alvares et al. 2013). We survey for amphibians in 11 sampling sites with four km long, installed in both banks of the Tapajós River and its tributary the Jamanxim River. Each sampling site covered humid primary *Terra Firme* forests, which does not suffer the seasonal riverine flood pulse effect (Junk et al. 1989) and riparian forests (Fig. 1). We used complementary sampling methods (Heyer et al. 1994), such as pitfall traps (600 trap nights) and diurnal and nocturnal active searches (more than 340 days). Six field campaigns were conducted along July 2012 and November 2013.

Aiming to better understand the relevance of these records in the general context of the geographic and elevational distribution of the species, we survey for its occurrence data available in the literature (Spix 1824; Myers and Carvalho 1945; Trueb 1974; Duellman and Lynch 1988; Rodríguez and Duellman 1994; Ruiz-Carranza et al. 1996; Sheil and Mendelson III 2001; Lehr 2001; Moravec et al. 2002; Coloma et al. 2004; Duellman 2005; Lynch 2005; Cisneros-Heredia 2006; Glaw and Franzen 2006; Muñoz-Saravia 2008; Souza 2009; von May et al. 2009; Beirne and Whitworth 2011; Bernarde et al. 2011; Catenazzi et al. 2013; Almendáriz et al. 2014; Castroviejo-Fisher et al. 2015; Frost 2017; Rainforest Conservation Fund 2017; AP Lima, pers. comm.) and online databases (Ortiz 2013; GBIF 2017; SpeciesLink 2017), mostly with associated vouchers in zoological collections, obtaining a total of 77 georeferenced localities of occurrence.

Morphologic data survey

We analyzed morphologic data traditionally used in the taxonomy of the genus (Trueb 1974), obtaining qualitative characters of external morphology and quantitative characters using a caliper to the nearest 0.1 mm: snout–vent length (SVL); forearm length from proximal edge of palmar tubercle to outer edge of flexed elbow (FAL); hand length from proximal edge of palmar tubercle to tip of finger III (HA); tibia length from proximal edge of

Fig. 2. Specimens of *Hemiphractus scutatus* from middle Tapajós River region, Pará State, Brazil. **(a)** Female, 76.1 mm SVL, INPA-H38116; **(b)** Male, 57.8 mm SVL, INPA-H38117; **(c)** Female, 61.7 mm SVL, INPA-H38118.

flexed knee to heel (TL); foot length from proximal edge of inner metatarsal tubercle to tip of Toe IV (FL); head width at level of angle of jaw (HW); head length from angle of jaw to tip of snout (HL); eye diameter (ED); internarial distance (IN); diameter of tympanum (DT); interorbital distance (IO) and thigh length (THL). We compared the measurements with information available from the literature and voucher specimens deposited at the Collection of Amphibians and Reptiles (INPA-H) of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil (Appendix S1), where the new specimens were also deposited (under accession numbers INPA-H 38116–38118).

Molecular data protocols

We extracted the genomic DNA from two specimens liver tissue samples conserved in absolute ethanol using the phenol-chloroform protocol (Sambrook and Russel 2001). The *16S* mtDNA gene, a standard marker for amphibians (Vences et al. 2012), was amplified via the Polymerase Chain Reaction (PCR). The PCR amplification used a mix with final volume of 25 µl: 4 µl of 1.25 M dNTPs, 2.5 µl of 10X amplification buffer (75mM Tris HCl, 50 mM KCl, 20 mM $(NH_4)_2SO_4$), 1.0 µl of 50 mM $MgCl_2$, 1.0 µl of DNA in a concentration of 250 ng/µl, 0.25 µl of each primer (*16S*ar and *16S*br - Palumbi et al. 1991) in a concentration of 200 ng/ul, 0.25 µl of Taq DNA polymerase 5 U/µL and 15.75 µL of ddH_2O. Reaction conditions had an initial heating step at 94 °C for five minutes, 30 cycles of denaturation at 94 °C for 30 s, primer annealing at 50 °C for 60 s, and extension at 72 °C for 120 s, followed by a final extension at 72 °C for seven minutes. PCR products were purified with ExoSAP-IT (USB Corporation) and submitted to a sequencing reaction following BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, EUA) protocols. The sequences were obtained in the automated sequencer ABI PRISM 3500 (Applied Biosystems, EUA) and deposited in GenBank (accession numbers MG011478, MG011479).

The sequences were aligned using the MUSCLE algorithm, implemented in MEGA 6.06 (Tamura et al. 2013) and corrected manually, obtaining a 524 bp alignment. Using the same software, we generated a maximum likelihood phylogenetic tree, constructed through a general time reversible model with a gamma distribution of rate variation (GTR+G), selected as the best DNA evolution model for the alignment by Bayesian Information Criterion (BIC), as well as to calculate two inter and intra-specific genetic distances: uncorrected-pairwise and Kimura-2-Parameter (K2P) (Kimura 1980). Additional sequences were obtained in GenBank, including the two distinct lineages of *H. scutatus* identified by Castroviejo-Fisher et al. (2015) (Table 3). The statistical support for the tree nodes was estimated by bootstrapping (5,000 replicates).

Results

New records of *Hemiphractus scutatus*

We found three specimens of *H. scutatus* in two of the 11 sampling sites (Figs. 1–3). It was a rare species in the sampling, recorded at a ratio of one specimen in about each 300 days of sampling, while the most abundant syntopic terrestrial frogs were from genera *Adenomera* Steindachner, 1867, *Pristimantis* Jiménez de la Espada, 1870, *Allobates* Zimmermann and Zimmermann, 1988, and *Rhinella* Fitzinger, 1826, with 2,700 specimens recorded in this same sampling effort. The three specimens of *H. scutatus* were only recorded by the active searches, and exclusively in *Terra Firme* forests (Fig. 4).

On 28 September 2012 one female voucher specimen was collected by D. Pavan close to a large tree and under a palm leaf, on the left bank of Tapajós River, at 19:15 h (76.1 mm SVL; 05°02'S, 56°53'W, 62 m above mean sea level, hereafter referred as asl). On 16 October 2012 a male voucher specimen was collected on the same riverbank by LJCL Moraes hidden inside the leaf-litter at 21:05 h, distant ca. 51 km in straight line from the first record (57.8 mm SVL; 04°39'S, 56°37'W, 60 m asl). On 28 April 2013 a second female voucher specimen was collected also hidden inside the leaf-litter on the same riverbank by J. Cassimiro at 21:30 h (61.7 mm SVL; 04°40'S, 56°37'W, 83 m asl), distant ca. 50 km in straight line from the first record and 430 m from the second record. No evidence of reproductive activity or gaping posture (Trueb 1974) was observed.

These three records represent the easternmost known localities of occurrence of *H. scutatus*, extending the geo-

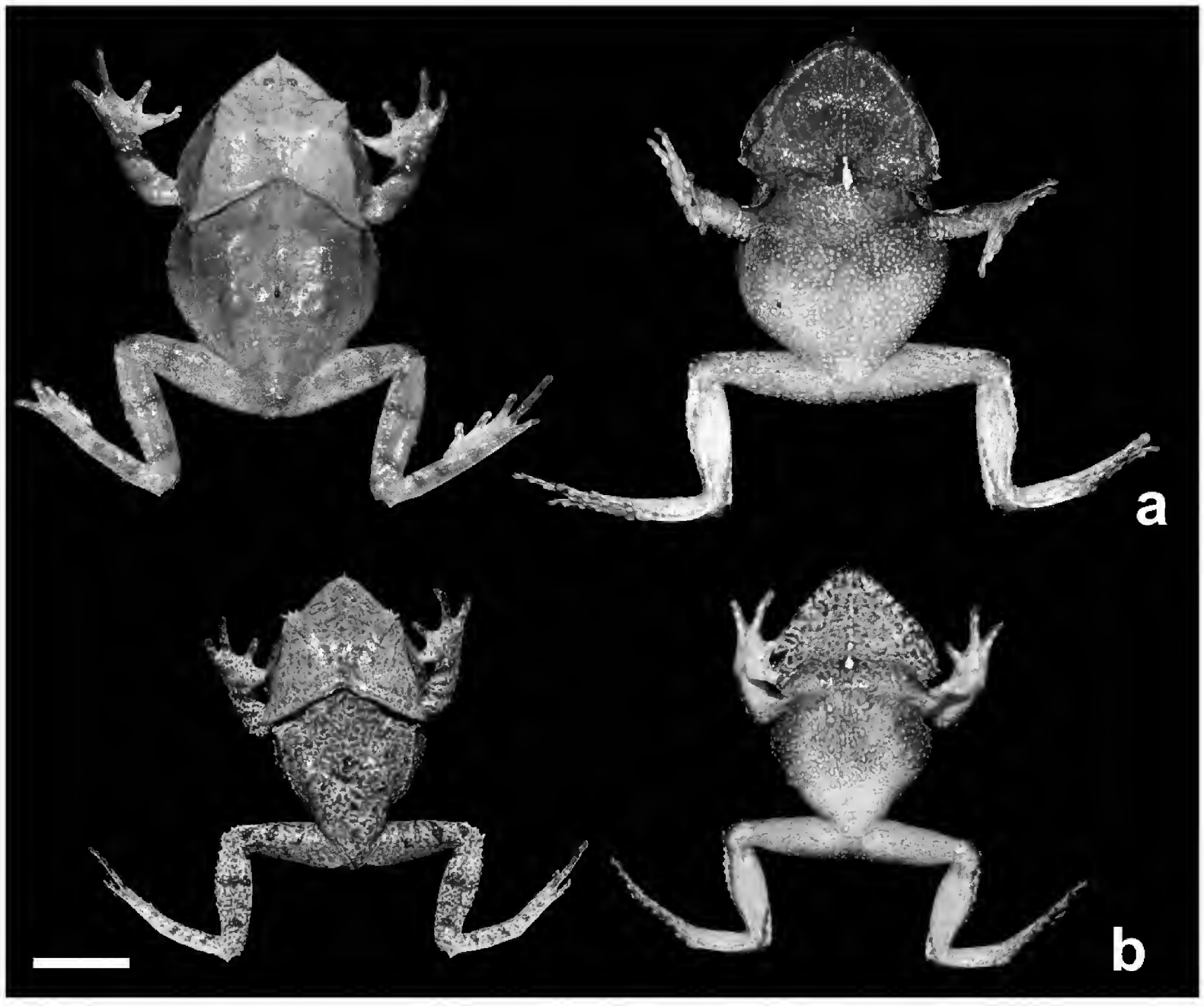

Fig. 3. Dorsal and ventral views of voucher specimens of *Hemiphractus scutatus* from middle Tapajós River region, Pará State, Brazil. **(a)** Female, 76.1 mm SVL, INPA-H38116; **(b)** Male, 57.8 mm SVL, INPA-H38117. Scale bar = 20 mm.

graphic range of the species and the genus *Hemiphractus*. They are distant ca. 1,000–1,500 km from the previously known easternmost points of the species occurrence, in Rondônia (INPA-H 15398, 15399) and Amazonas States, Brazil (GBIF 2017; SpeciesLink 2017) (Fig. 1). Considering only the Amazon Basin at South of Amazon River, these new records even extend to the East the geographic range of the family Hemiphractidae. Furthermore, the elevation level in which these specimens were recorded are among the lowest known elevation for the species (60, 62, and 83 m asl; Fig. 5), and two of them (60 and 62 m asl) also extend downwards the known elevational range of this species, since there are no documented records of individuals below 70 m asl.

Morphologic variation and molecular relationships

The morphologic data confirms the identification of our specimens in accordance to the literature (Trueb 1974) and voucher specimens. Qualitative characters include the triangular head, canthus rostralis rounded in section; tympanum large and vertically ovoid; oblique rows of tubercles on dorsal surfaces of forearm and hind limb (less pronounced in female specimens); small triangular fleshy proboscis, dorsoventrally flattened, on tip of snout; eyelids granular with one (female specimens) or three (male specimen) prominent fleshy conical tubercles; single bony projection at the angle of the jaw; slightly enlarged tubercles at the knee and small tubercles at calcaneum (divergent from the absence of calcar projections reported by Trueb 1974 and Rodríguez and Duellman 1994); fingers and toes with vestigial adhesive discs, well-developed round subarticular tubercles and basal webbing; thenar tubercle elliptical and outer palmar tubercle diffuse, flat and cordiform; no evidence of nuptial pads in male specimen; toes also with well-developed round subarticular tubercles and about one-fourth webbed; inner metatarsal tubercle well-developed and elliptical, and outer metatarsal tubercle indistinct; shagreened skin on dorsum and granular on flanks, abdomen and ventral surfaces of thighs.

Dorsal coloration in life varies from reddish brown (INPA-H38116 and 38118) to pale tan background with dark mottling (INPA-H38117), with two dark vertebral spots; dark suborbital marks from the lower margin of the eye expanding posteroventrally but not reaching the lip (more pronounced in INPA-H38117 than in INPA-H38116 and 38118) and scattered dark spots in the tympanic region. Ventrally, gular coloration varies from uniformly brown (INPA-H38116 and 38118) to mottled (INPA-H38117), with a pale mid-ventral stripe reach-

Fig. 4. Aerial **(a)** and inside **(b)** view of the *Hemiphractus scutatus* habitat (*Terra Firme* forest) in middle Tapajós River region, Pará State, Brazil, also showing the BR-230 highway.

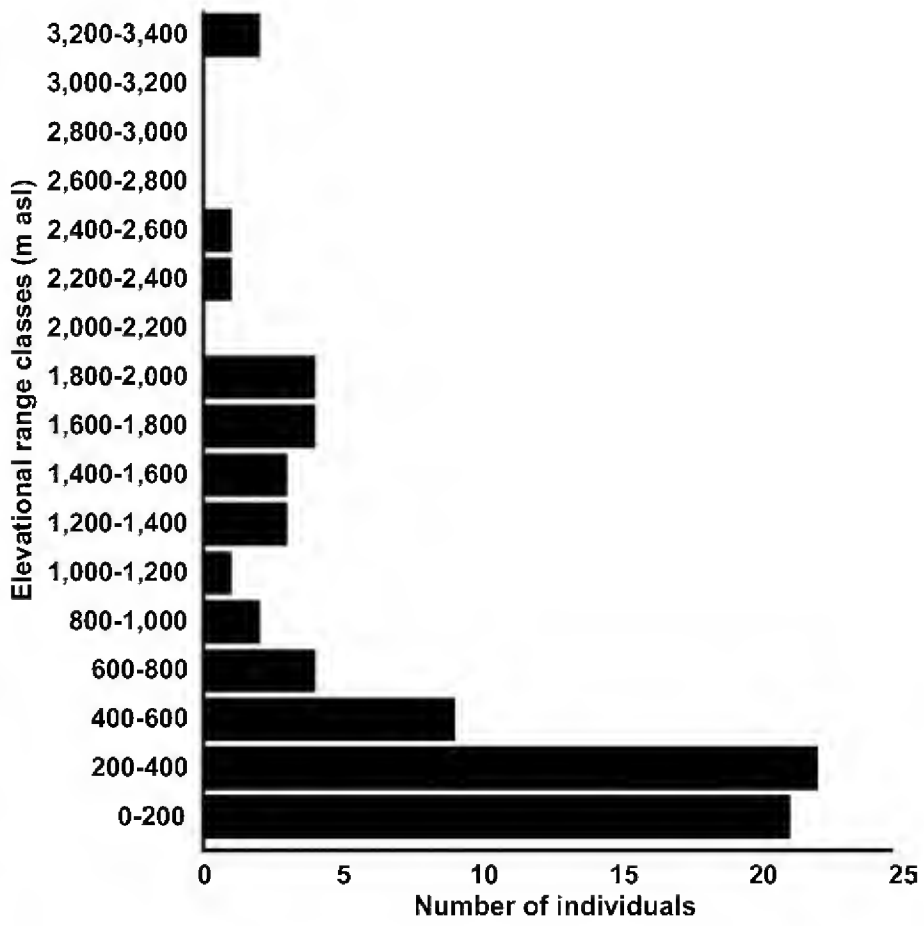

Fig. 5. Variation in number of individuals of *Hemiphractus scutatus* recorded along its known elevational range (60–3,300 m above mean sea level). The specimens from middle Tapajós River region, Pará State, Brazil are recorded among the lowest known elevation for the species.

ing the pectoral region; same gular color reaches the pectoral region, and becomes less pigmented posteriorly. A finely dark venate pattern covers the flank areas above the forelimb; forelimbs and hind limbs varies from uniformly brown (INPA-H38116 and 38118) to tan (INPA-H38117), with dark transverse bands, reaching the dorsal surface of hands (more evident in INPA-H38117); iris bronze and darker ventrally, with a longitudinally crossing reddish area and pupil horizontal. Regarding quantitative characters, most of the measurements of the middle Tapajós River specimens agree with the known morphometric range of the species (Table 1), also showing the sexual dimorphism in body size. The only divergence is a small HW compared to SVL in female INPA-H38116.

The *16S* mtDNA tree for *Hemiphractus* species shows, as the results presented by Castroviejo-Fisher et al. (2015), two distinct lineages of *H. scutatus*. The middle Tapajós River population is more related to the lineage from Peru (Figs. 6, 7), as the sequences have a higher genetic similarity (more than 97%) compared to sequence from Colombia, near the country's border with Brazil (93%) (Fig. 7).

Discussion

The presence of possible cryptic taxa under the name *H. scutatus* was suggested based on the results of a phylogeny of molecular and morphologic data (Castroviejo-Fisher et al. 2015), since two genetically distant intraspecific lineages were found. Although we initially worked with the hypothesis that specimens from middle Tapajós River were a new taxon, the morphologic and molecular analysis readily rejected this. Regarding the morphology, despite the possibility of strong variation due to large geographic distance to known distribution area, most of the qualitative and quantitative data of the specimens from middle Tapajós River were inside the known range for the species (Trueb 1974; Rodríguez and Duellman 1994) and other voucher specimens (Table 1). The slightly divergences in colors, shapes, and morphometric characters between this specimens and the known for the species may be part of intraspecific variation. Regarding the molecular data, despite the high geographic distance between the populations from middle Tapajós River and Peru (more than 2,300 km), there is a low genetic distance between the sequences from these regions (between 2% and 3%). As Castroviejo-Fisher et al. (2015) highlighted, the genetic distance between the sequences from Colombia and Peru, and now including the distance of Tapajós sequences, are high and may indicate cryptic speciation (more than 7%). As overall similarity in external morphology and pronounced morphologic variation are common events inside the genus *Hemiphractus* (Trueb 1974), further broader studies and integrative taxonomic revisions may indicate the extent of morphologic and molecular variability of this species and reveal the taxonomic status of these genetically distant lineages.

Table 1. Morphologic measurements (mm) of *Hemiphractus scutatus* specimens recorded in middle Tapajós River region (highlighted), compared to literature data and other voucher specimens from herpetological collection of Instituto Nacional de Pesquisas da Amazônia, Brazil (INPA-H). Literature: [a]Trueb 1974, $n = 8$ males and 15 females; [b]Rodríguez and Duellman 1994.

Measurements	Literature	INPA-H38117	Literature	INPA-H15399	INPA-H15398	INPA-H38116	INPA-H38118
	Male	Male	Female	Female	Female	Female	Female
SVL	36.9–62[a,b]	**57.8**	60.4–81[a,b]	62.5	73.6	**76.1**	**61.7**
FAL	-	**12.7**	-	12.2	15.7	**16.4**	**12.9**
HA	-	**16.3**	-	18	21.3	**19**	**17.6**
TL	15.5–27.5[a]	**25.1**	23.7–38.3[a]	21.2	34.5	**32**	**27.6**
FL	-	**26.2**	-	25.5	32	**30.1**	**26.7**
HL	17.5–30[a]	**28.3**	25.7–42.9[a]	29	33	**36.3**	**31**
HW	20.8–37.5[a]	**34.2**	34.4–52.8[a]	36	43.3	**41.6**	**37.5**
ED	-	**6**	-	5.6	7.3	7	**5.6**
IN	-	**4**	-	3.8	4.5	**4.8**	**4**
DT	-	**5.2**	-	3.3	4.9	**4.7**	**3.7**
IO	-	**14.6**	-	14.4	17.6	**17.8**	**17.3**
THL	-	**28.2**	-	29.7	38.2	**34.4**	**29.2**
TL/SVL (%)	42.3–48[a]	**43**	39.3–47.6[a]	34	47	**42**	**45**
HL/SVL (%)	47.6–52.3[a]	**49**	42.7–53.3[a]	46	45	**48**	**50**
HW/SVL (%)	56.6–65.5[a]	**59**	57.1–65.7[a]	58	59	**55**	**61**

Biogeography

After more than 190 years since the original description of *H. scutatus* (Spix 1824) we recorded this species in the eastern Amazonia, emphasizing the lack of knowledge about the general biogeographic patterns of Amazonian amphibians, which can be mainly generated by sampling difficulties, especially in cases of secretive species. Large forested regions in the Amazonia remain unexplored and have the potential to harbor new species or expanding species distributions (Azevedo-Ramos and Galatti 2002). Therefore, the recognition of broader biogeographic patterns to Amazonian amphibians, as areas of endemism historically recognized in the biome to other vertebrates (e.g., Cracraft 1985; Boubli et al. 2014) depends on the continued expansion of the sampling effort and new analitical techniques that is currently being held in the biome.

Our new records for *H. scutatus* bring new information to a biogeographic idea historically recognized on the low representation of Hemiphractidae in the eastern Amazonia, probably due to increased seasonality in this region (Sombroek 2001; Duellman 2015). Species of this family that have greater environmental plasticity, as appears to be the case of *H. scutatus* (the species of the genus with the widest known geographic and elevational range) may reach the preserved forests in this region and establish viable populations, although in less abundance in relation to the more climatically constant (Wang et al. 2017) and humid environments of western Amazonia.

Regarding elevational occurrence, although *H. scutatus* has already been recorded in high elevations at the Andean mountain range (GBIF 2017), a greater number of individuals is known for the Amazonian lowlands, and 67% of 77 published localities of occurrence are below 600 m asl (Fig. 5). This wide elevational range reinforce the high environmental plasticity, as the life-history strategies of amphibian populations in high and lowlands may drastically differ (Morrison and Hero 2003). The knowledge on the drivers of elevational variation in the distribution of Amazonian amphibians is still incipi-

Table 2. Sequences from GenBank with accession numbers. In bold are sequences generated from this study.

Taxon	*16S*
Hemiphractus bubalus	DQ679412
Hemiphractus fasciatus	KC014899
Hemiphractus fasciatus	KC014900
Hemiphractus fasciatus	KC129336
Hemiphractus fasciatus	KC129337
Hemiphractus fasciatus	KC129338
Hemiphractus fasciatus	KC129339
Hemiphractus fasciatus	KC129340
Hemiphractus fasciatus	KC129341
Hemiphractus fasciatus	KC129342
Hemiphractus fasciatus	KC129343
Hemiphractus helioi	AY843594
Hemiphractus helioi	KR270431
Hemiphractus proboscideus	DQ679413
Hemiphractus scutatus	DQ679414
Hemiphractus scutatus	KR270432
Hemiphractus scutatus	**MG011478**
Hemiphractus scutatus	**MG011479**

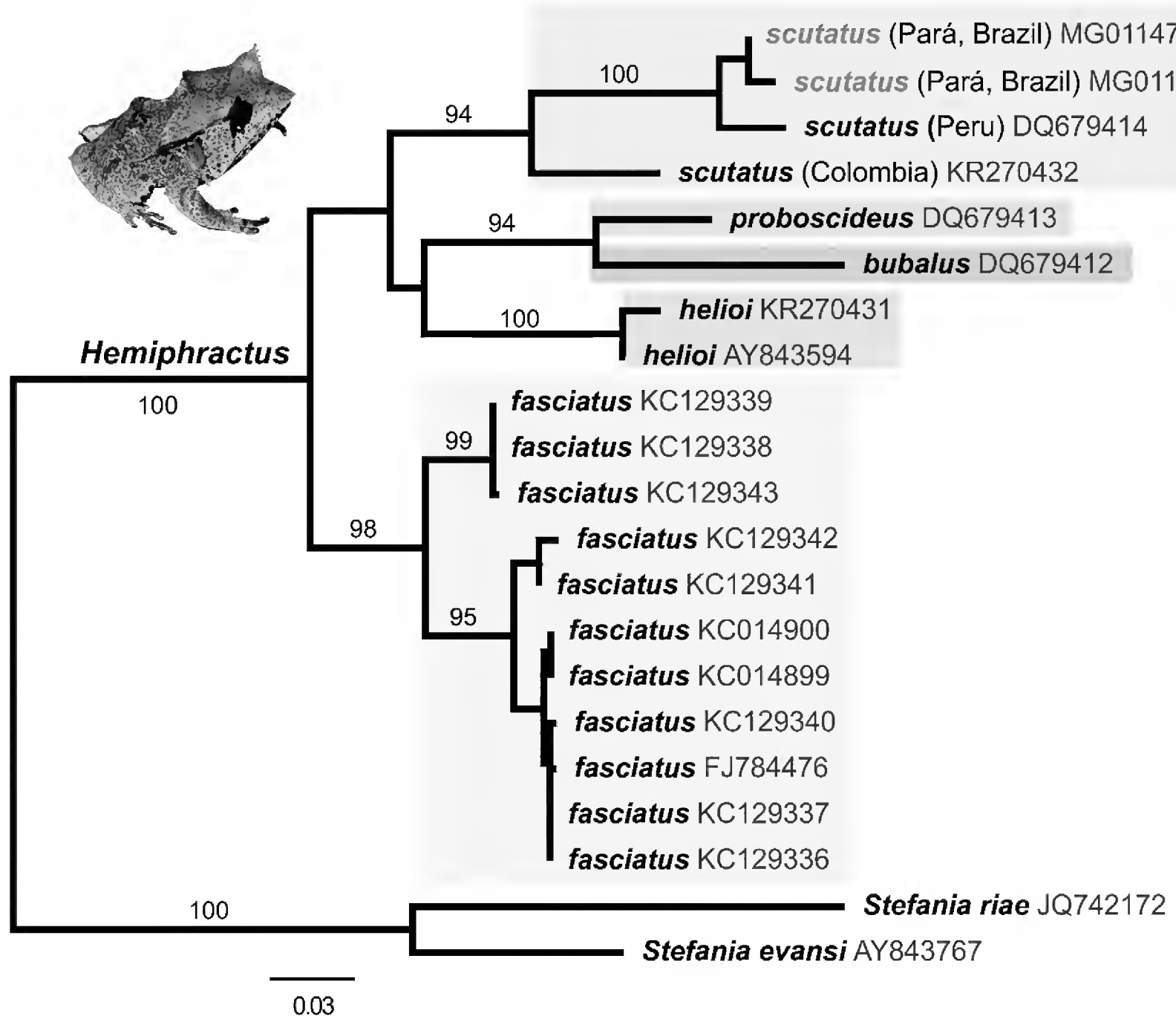

Fig. 6. Maximum likelihood phylogenetic tree of *Hemiphractus* species based in a fragment of the *16S* mtDNA gene, with GenBank accession numbers. Only bootstrap values >80% are shown (5,000 replicates). For *Hemiphractus scutatus*, sample localities are in parentheses and specimens from middle Tapajós River region, Pará State, Brazil are highlighted.

ent (Siqueira and Rocha 2013) and the *H. scutatus* may be a target taxon for future studies testing this gradient.

Conservation

Hemiphractus scutatus is considered as "Least Concern" by IUCN due to its wide distribution and presumably large and stable populations (Coloma et al. 2004). However, this species is rarely recorded and have poorly known population dynamics to define its conservation status, that can even vary along its wide geographic and elevational range. As the Amazon region has suffered increasing anthropic impact through the advance of cities and highways, forests fragmentation and habitat loss (Fearnside 2015), the *H. scutatus* may have declining populations in most of its distribution, since they are dependents of undisturbed forests (Rodríguez and Duellman 1994).

The specimens of *H. scutatus* from middle Tapajós River region may represent a unique population, recorded near and within a federal conservation unit (Parque Nacional da Amazônia), same pattern already described for Peruvian populations (von May et al. 2009), reinforcing the need to maintain large protected forest areas in the Amazonia and adequate land-use on the unprotected (Laurance et al. 2001). In addition to these threats, the biome has been target of dam construction in its larger rivers (Latrubesse et al. 2017), which can negatively affect the biodiversity of the surrounding forests (Moraes et al. 2016). The population of *H. scutatus* from Tapajós River is in the region affected by the construction of a large hydroelectric plant, part of a complex planned for the basin (Fearnside 2015), thus the implementation of this project may affect the viability of this population.

Conclusion

The discovery of the first specimens of *H. scutatus* from eastern Amazonia sheds new insights into ecology, biogeography, taxonomy, and conservation of these remarkable frogs. To better understand the population status and the total distribution range of this taxon in Amazonia, we need more long-term field studies, with standardized protocols, complementary sampling and broader approaches.

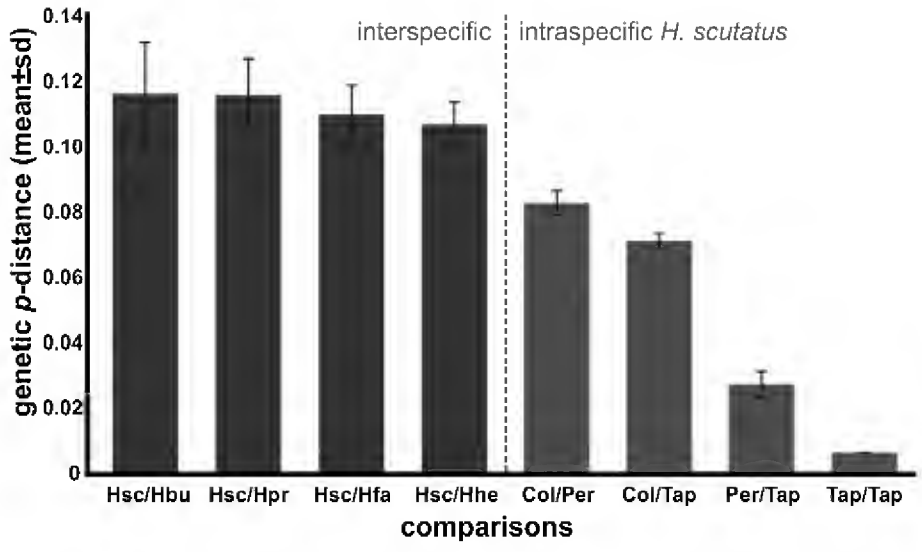

Fig. 7. Inter and intraspecific genetic distances (mean ± standard deviation of pairwise and K2P distances) calculated for a fragment of *16S* mtDNA gene of *Hemiphractus* species and populations. (Hsc) *Hemiphractus scutatus*; (Hpr) *Hemiphractus proboscideus* (Hfa) *Hemiphractus fasciatus*; (Hhe) *Hemiphractus helioi*; (Col) Colombia; (Per) Peru; (Tap) middle Tapajós River region, Pará State, Brazil. GenBank accession numbers of sequences are in Table 2.

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Appendix S1.

Specimens examined. *Hemiphractus scutatus* (*n* = 5): BRAZIL: Rondônia: Abunã esquerda, Porto Velho (65°20'S 09°31'W), INPA-H15398, Jirau esquerda, Porto Velho, INPA-H15399 (64°44'S 09°20'W); Pará: Left bank of middle Tapajós River, Itaituba (05°02'S 56°53'W), INPA-H38116, Left bank of middle Tapajós River, Itaituba (04°39'S 56°37'W), INPA-H38117. Left bank of middle Tapajós River, Itaituba (04°40'S 56°37'W), INPA-H38118. INPA-H = Collection of Amphibians and Reptiles of the Instituto Nacional de Pesquisas da Amazônia, Manaus, AM, Brazil.

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Sighting of the Himalayan Trinket Snake, *Orthriophis hodgsonii* Günther (1860) (Reptilia: Colubridae), in Sahastra Dhara, Uttarakhand: A new elevational record

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***Abstract.*—In 2016, two individuals of *Orthriophis hodgsonii* (Himalayan Trinket Snake) were observed from the Sahastra Dhara area, Uttarakhand, India, confirming the occurrence of this species in the Garhwal region. This report provides the lowest elevational record (835 m) of *Orthriophis hodgsonii* from its previously known distribution range (1,000–3,200 m).**

Keywords. Geographic distribution, north India, Garhwal region, range extension, Sauria, conservation

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Of the four species in the genus *Orthriophis* found across the world (*Orthriophis mollendorffi*, *O. taeniurus*, *O. hodgsonii*, and *O. cantoris*), three are found in India (*O. taeniurus*, *O. hodgsonii*, and *O. cantoris*) (Utiger et al. 2002; Whitaker and Captain 2004). The Himalayan Trinket Snake (*Orthriophis hodgsoni*) is native to India, Nepal, and China (Tibet) (Whitaker and Captain 2004). In India, this species is distributed in Jammu and Kashmir northern Punjab, Himachal Pradesh, Uttarakhand, Bihar, Northern, West Bengal Sikkim and Meghalaya, ranging from the elevation of 1,000–3,200 m (Smith 1943; Das 2002; Whitaker and Captain 2004; Sharma 2007).

On 1 April 2016 (11:10 hours), a Himalayan Trinket Snake (sex unknown) was recorded from a small barren plot, located close to human settlements in the Sahastra Dhara area near Dehradun (30°23′07.4″N, 78°07′40.5″E, 831.4 m; Fig. 1). The spot was near the protected forest of the Mussoorie Forest Division. Some of the plants in this area where the snakes were seen are: *Murraya koenigii* (Curry Tree), *Lantana camara* (Lantana), *Jatropha curcas* (Ban Arandi), and *Datura stramonium* (Jimson Weed). Both snakes (including the one recorded below) were photographed and visually identified based on descriptions given by Smith (1943), Sharma (2007), and Whitaker and Captain (2004). No scalation data was recorded. Thereafter, on 18 August 2016 (13:20 hours), an individual of unknown sex was observed far from where the first specimen was recorded (30°23′05.5″N, 78°07′44.6″E, 839.4 m; Figs. 2 and 3). Both sightings were during the summer season and near the Sahastra Dhara.

Husain and Ray (1995) first recorded this species from Pauri, Chamoli, and Nainital districts of the Uttarakhand State. Thereafter, Whitaker and Captain (2004) recorded this species from Mussoorie, Almora, and Nainital districts of Uttarakhand State, at an elevation ranging from 1,000–3,200 m. Smith (1943), Sharma (2003) and Bahuguna (2010) also corroborated the presence of the Himalayan Trinket Snake in the State. Vasudevan and Sondhi (2010) had only included the Himalayan Trinket Snake in a checklist of snakes of Uttarakhand but no description and locality records were stated.

We herein confirm the occurrence of the Himalayan Trinket Snake in the Uttarakhand State, Garhwal region (Sahastra Dhara–a perennial river) and provide the first record of its presence below 1,000 m. The Sahastra Dhara is located in northern India at 29°26'–31°28'N and 77°49'–80°06'E and falls within the Himalaya Biogeographic Zone and located in the West Himalaya Province. The largest portion of this area is in the Shivalik's Biogeographic Subdivision, which constitutes an important repository of reptilian fauna.

The Himalayan Trinket Snake has not yet been assessed by the IUCN Red List. Increasing development

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Fig. 1. Himalayan Trinket Snake, *Orthriophis hodgsonii*, near Sahastra Dhara.

Fig. 2. Close-up of the head of a Himalayan Trinket Snake recorded from Sahastra Dhara.

and anthropogenic activities across the riparian corridors, shrinkage of natural water sources inside protected areas, expansion of the road network across a long chain of protected habitats, and lack of awareness among the local people were some of the observed threats that could potentially lead to population decline of the species.

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Fig. 3. Himalayan Trinket Snake in its natural habitat in the Sahastra Dhara area.

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Captive management, reproduction, and comparative larval development of Klappenbach's Red-bellied Frog, *Melanophryniscus klappenbachi* Prigioni and Langone, 2000

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***Abstract.*—In this study, we report on the successful keeping, breeding, and rearing of Klappenbach's Red-bellied Frog, *Melanophryniscus klappenbachi* Prigioni and Langone, 2000. Breeding and spawning took place after a relatively dry period without using a brumation period. To initiate mating behavior the toads were introduced into a rain chamber with a raised water level and constant irrigation in accordance with the toad's natural habitat and heavy rainfalls. The fast developing tadpoles started metamorphosis after 19 days at a constant water temperature of 23 °C and pH values between 6.5 and 7.9. A higher pH value led to slightly faster growth irrespective if tadpoles were reared singly or in groups.**

Keywords. Amphibians, Anura, captive breeding, conservation breeding, environmental factors, spawning

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Introduction

The genus *Melanophryniscus* Gallardo, 1961 is currently represented by 29 species which have been reported from southern Bolivia and southern Brazil in the north over Paraguay to Uruguay and northern Argentina in the south. They are commonly referred to as South American Redbelly Toads due to the red or orange flash markings upon their ventral bodies, hands, and feet (Frost 2016). Species of this genus have been divided into three phenotypic species groups based on morphological characteristics: the *Melanophryniscus tumifrons*, *M. moreirae*, and *M. stelzneri* groups (Cruz and Caramaschi 2003). Klappenbach's Red-bellied Frog (*M. klappenbachi* Prigioni and Langone, 2000) is part of the *Melanophryniscus stelzneri* group (Cruz and Caramaschi 2003), which currently includes eight more species, i.e., *M. atroluteus* (Miranda-Ribeiro, 1920), *M. cupreuscapularis* (Céspedez and Alvarez, 2000), *M. dorsalis* (Mertens, 1933), *M. fulvoguttatus* (Mertens, 1937), *M. krauczuki* (Baldo and Basso, 2004), *M. montevidensis* (Philippi, 1902), *M. rubriventris* (Vellard, 1947), and *M. stelzneri* (Weyenbergh, 1875).

Klappenbach's Red-bellied Frog is characterized by a yellow stripe between the eyes or two to three large yellow blotches forming a distinct interocular band. Its dorsal and ventral surfaces are covered with small and large, irregularly formed yellow blotches on a black base color (see Fig. 1A and B) (Kwet et al. 2005). Adults of this diurnal species reach an average size of 2.5 to 3.0 cm (Prigioni and Langone 2000). *Melanophryniscus klappenbachi* inhabits usually dry environments, such as shrubland habitats, in north-eastern Argentina and Paraguay at 50 to 100 m above sea level (Bland 2015; Frost 2016). After heavy rainfalls explosive breeding takes place in the emerging ephemeral water bodies (Aquino et al. 2004). Fast development of tadpoles increases the probability of completing metamorphosis before water bodies dry out (Kurth et al. 2013).

Currently Klappenbach's Red-bellied Frog is listed as Least Concern by the IUCN Red List of Threatened Species, due to its wide distribution, large and stable populations, and its tolerance for habitat modification (Aquino et al. 2004).

Although it was once a popular species in the pet trade it does not seem to be regularly reproduced in captivity (Bland 2015; Aquino et al. 2004). Amphibians are one of the most threatened animal taxa with more than one third of the currently described species (ca. 7,520) recognized as threatened with extinction (Frost 2016; Hoffmann et al. 2010; Stuart et al. 2004). For threatened species *ex situ* captive-breeding programs are relevant instruments

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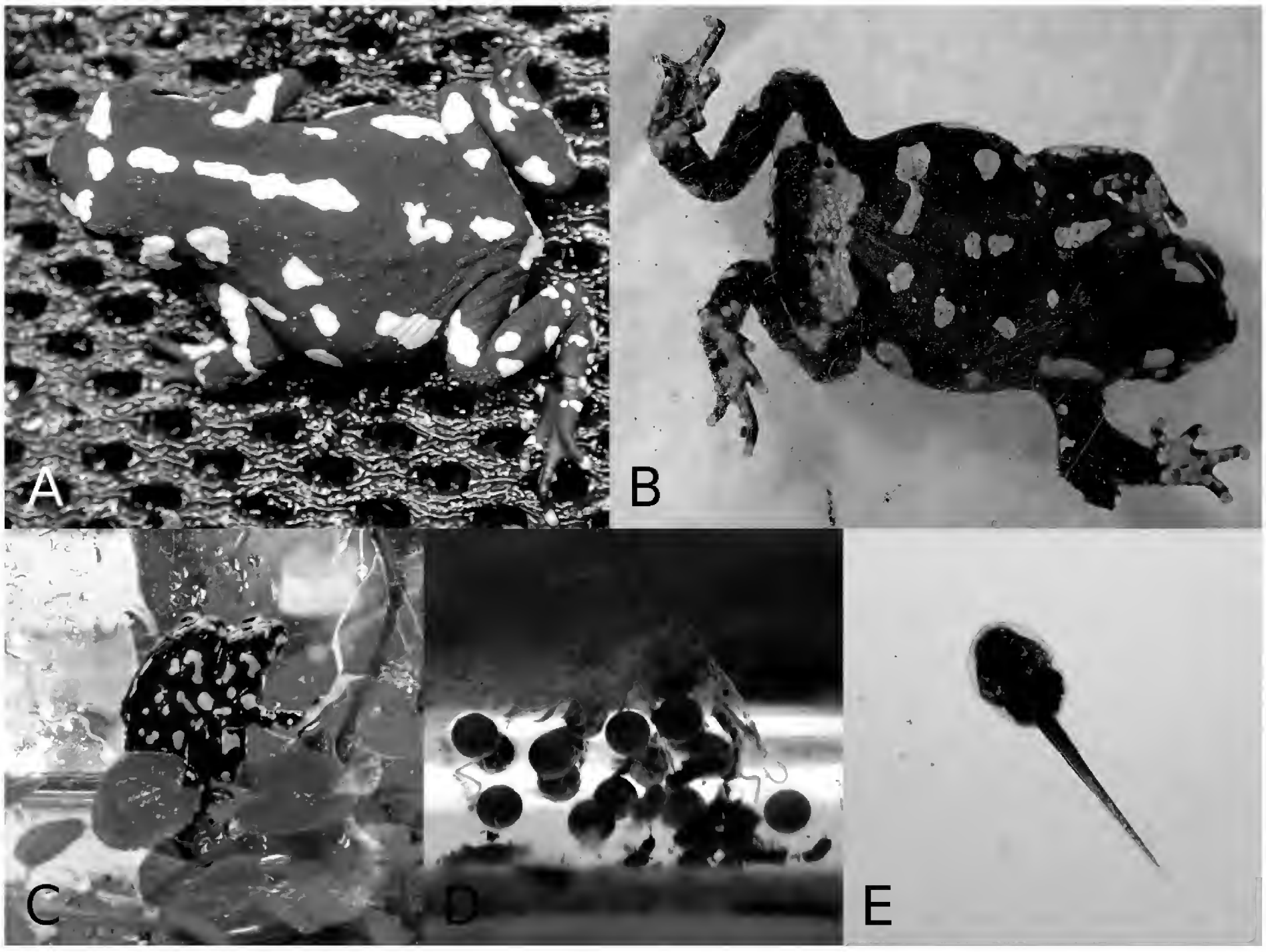

Fig. 1. *Melanophryniscus klappenbachi*. **(A)** Dorsal and **(B)** ventral view of an adult female. **(C)** Amplexus. **(D)** Egg clump attached to moss. **(E)** Contrasting photo of a tadpole, used for evaluating the growth.

in learning more about a species and to build up assurance populations against extinction (Gawor et al. 2011).

Kurth et al. (2013) have already reported on reproductive cues and larval development in *M. klappenbachi*, while Bland (2015) has given a short report of rearing captive bred *M. klappenbachi*. However, both papers did not consider influences of differing water parameters or group sizes on the development of young tadpoles.

Herein, we present captive management conditions at the animal keeping facility of the Zoological Research Museum Alexander Koenig (ZFMK), Bonn, Germany, experiences in breeding without the use of a brumation period, and rearing tadpoles. Further this paper reports on the influence of different pH values and group sizes for growth and mortality rates of early developmental stages of Klappenbach's Red-bellied Frog.

Materials and Methods

Captive management and breeding

The basis for the breeding stock used in this study was built up by a group of eight *M. klappenbachi* purchased from a pet shop in 2011, which were imported from Paraguay according to the vendor. The group of adult toads were housed in a terrarium measuring 120 × 50 × 50 cm (L × W × H) in the animal keeping facility of the ZFMK. The terrarium bottom was covered with a six cm thick filter pad which was diagonally cut in the front, resulting in a water surface of 120 × 12 cm. There was at least a water level of two cm depth in the tank at all times, including the dry phase. Previous setups have revealed that toads were not able to swim for long periods of time, thus water levels needed to be shallow or a number of aquatic plants provided to prevent drowning. The complete ground and all three side walls were covered with Hygrolon®, a novel synthetic material that is non-decomposable and mimics the features of dead cellulose cells. This material is highly hygroscopic and used to ensure high air humidity.

The terrarium setup for the frogs was automatically misted three times per day for 30 seconds and air humidity varied between 70% and 80%. The setup was equipped with different plants, i.e., *Ficus pumila*, *Begonia* sp., *Neoregelia schultesiana*, *Pilea* sp., an undefined fast-growing Venezuelan tendril, and different mosses. Additionally leaf litter, pieces of cork bark, and roots were added to the terrarium in order to provide hiding and climbing space for the toads. Photoperiod was set to daylight between 8:00 and 20:00 h, as lighting LED light

Fig. 2. Keeping and rearing *M. klappenbachi*. **(A)** Terrarium of the adult group housing eight specimens. **(B)** Rearing of the tadpole test groups in a climate chamber. **(C)** Rearing containers for the young toadlets.

strips (Solar Stinger 1,100 mm Sunstrip Dimmable Driver). Temperatures varied between 22 °C and 26 °C. Toads were fed flightless fruit flies (*Drosophila melanogaster* and *D. hydei*) and rarely with pinhead crickets (*Acheta domestica* and *Gryllus assimilis*). All prey was dusted with mineral or vitamin powder (i.e., herpetal Amphib, herpetal Mineral + Vitamin D3, and herpetal Complete Terrarium) and gut loaded with fresh vegetables or fruit puree before being fed to the toads.

To artificially initiate breeding season toads were placed into a second terrarium measuring 60 × 50 × 70 cm. The breeding terrarium was filled with water to a depth of six to seven cm and equipped with a few pieces of xaxim, a big root, and some plants (i.e., *Spathipyllum* sp., some mosses, and *Microsorum pteropus*) to provide small areas above the water. Temperature and lighting were the same as described above. In this tank a permanent rain system was installed that irrigated the back wall of the terrarium all day long. After successful mating and spawning, when the males stopped calling and the pairs did not exhibit amplexus any longer, all toads were transferred into the first terrarium again.

The spawn was divided into four aquariums measuring 30 × 30 × 30 cm filled with osmosis water to a depth of about 20 cm. Water temperature varied between 22 °C and 25 °C. A few aquatic plants (Java Fern, *Microsorum pteropus*) and small aquatic snails (Bladder Snails, *Physella* sp., and Ramshorn Snails, *Planorbella* sp.) were added into each aquarium.

Comparative setup

For rearing tadpoles water with various pH values was used: osmosis water with a measured pH of 6.5–7.0 and pond water with a measured pH of 7.7–8.0. To obtain water with a reduced pH value a small amount of peat was added to the osmosis water, until a pH of 5.5–5.8 was obtained. Afterwards water was filtered to remove the remaining peat. Table 1 lists the additionally measured water parameters.

Once tadpoles had hatched, a total of 108 larvae were randomly chosen and divided into different groups. All tadpoles were transferred into plastic boxes measuring 10 × 10 × 10 cm filled with a water depth of eight cm. Every box was equipped with a single aquatic plant (either a small Java Fern, *Microsorum pteropus*, or a short branch of Hornwort, *Ceratophyllum* sp.) to provide shelter and one aquatic snail (either Bladder Snails, *Physella* sp., or Ramshorn Snails, *Planorbella* sp.) to remove remaining food. For each pH value there were two different group sizes of either one tadpole or five tadpoles per box, to determine if group size influenced individual growth rate. Six samples were set up for each group size, so that a total of twelve boxes and 36 tadpoles were exposed to each pH value.

All tadpoles were kept in a climate chamber (Versatile Environmental Test Chamber MLR-352H-PE) under standardized conditions. The temperature was set to 23 °C and the photoperiod was set to daylight between 6:00 and 18:00 h each day. Inside the climate chamber boxes of different group sizes and pH values were placed randomly (see Fig. 2 B). The larvae were fed every second day with a mixture of pulverized fish food dissolved in water. Additionally one object slide overgrown with a thin layer of algae was placed in every box and renewed every three days. Two thirds of entire water content was exchanged every second day. All boxes were checked daily to remove deceased tadpoles and later to transfer metamorphosed froglets to a terrestrial setup.

Newly morphed toads were relocated into plastic containers measuring 33 × 21 × 28 cm. Two layers of Hygrolon® were used as ground layer to ensure high air humidity in rearing containers. One side of the boxes was placed on a heightened surface, so a height difference of 10 cm was formed from one end of the box to the other and a water part with a depth of 1–2 cm was created (see Fig. 2 C). As a result, a humidity gradient was created in the box, letting toads choose their preferred humidity. Containers were equipped with a small plant (undefined Venezuelan tendril) and some oak leaf litter.

Table 1. Water parameters of the different pH value groups.

	NO_2 [mg/L]	NO_3 [mg/L]	NH_4 [mg/L]	Cu [mg/L]	KH [°dH]	GH [°dH]	pH
Osmosis water	<0.05	7.5	<0.05	<0.1	3	5	6.5–7.0
Pond water	<0.05	<0.5	<0.05	<0.1	9	8	7.7–8.0
Peat water	<0.05	7.5	<0.05	<0.1	3	5	5.5–5.8

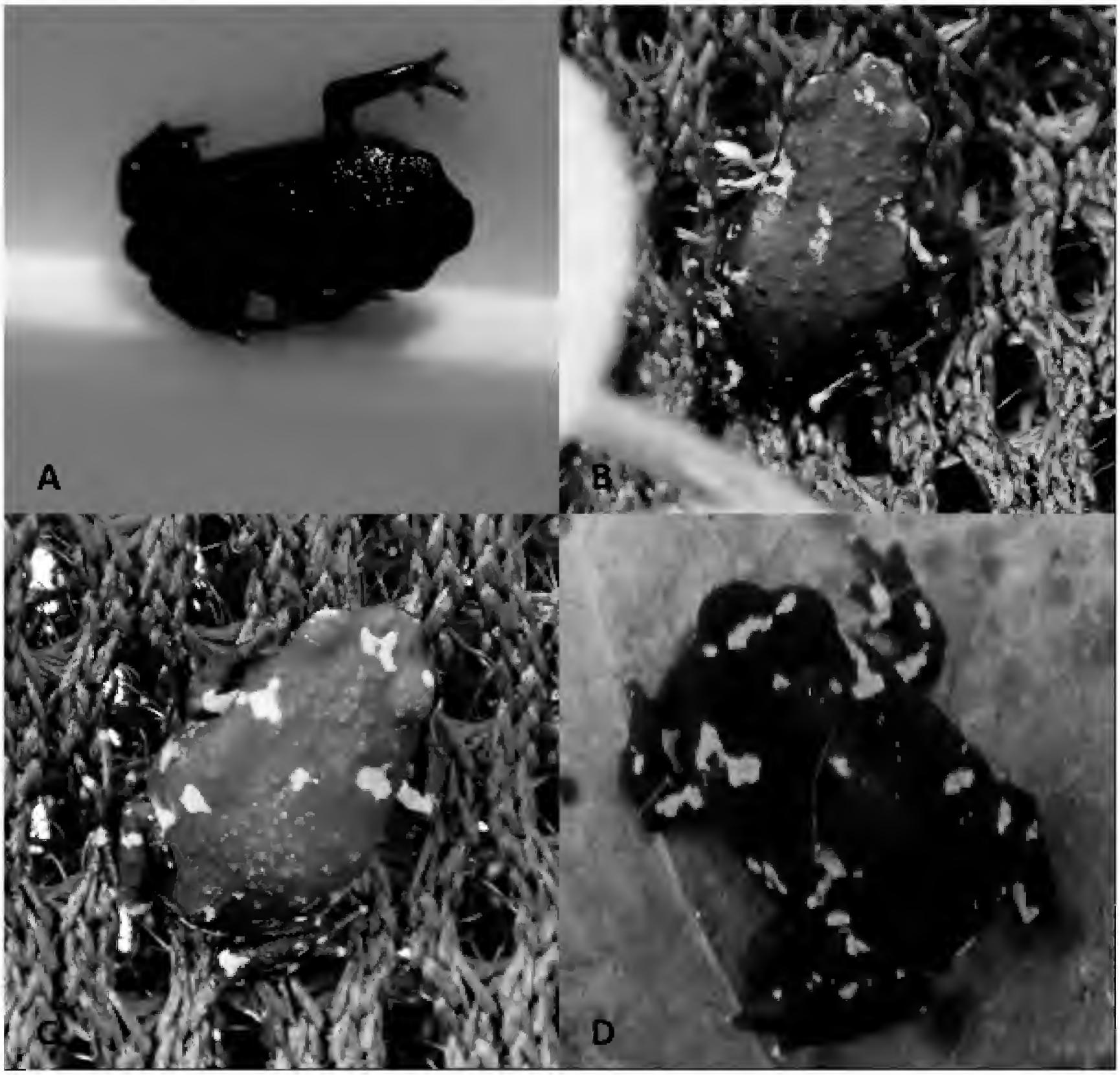

Fig. 3. Developing coloration in young toadlets of different ages. **(A)** Recently metamorphosed toadlet. **(B)** Ten days after metamorphosis. **(C)** Twenty-three days after metamorphosis. **(D)** Two months after metamorphosis.

Data collection and evaluation

Photos of each single tadpole were taken three times: first, on the day they were transferred into the boxes, second, five days later and third, one week later when the first tadpoles had already grown hind legs. Photos were taken with a digital camera (Olympus TG-2). Tadpoles were individually transferred into a petri dish placed on a transparent glass plate lightened from below thus increasing the contrast between tadpole and its surroundings (see Fig. 1 E).

To evaluate the growth of tadpoles the digital image analysis tool SAISAQ (Kurth et al. 2014) was used. This tool is programmed on the open source statistics platform R (R Developmental Core Team 2016) and facilitates the semiautomatic processing of standardized image files computing the surface area of a tadpole, which is highly correlated with its body mass (Kurth et al. 2014). As this method is non-invasive it is ideally suited for repeated measurements on live animals.

Results

Breeding

Within one hour after relocating adult toads into the breeding terrarium males began to call. The first amplexus could be observed only a few hours after relocating toads. Klappenbach's Red-bellied Frogs show an axillary amplexus. Eggs were found on the second day in the breeding tank. Spawn was mainly deposited in clumps of 10–30 eggs, some clumps were attached to the plants or xaxim pieces under water and some were just deposited in the water without any such attachment. Often next to egg clutches were a few single eggs. Eggs were greyish and had a diameter of 1.5–2.0 mm surrounded by a gelatinous capsule. The last recorded spawn was produced on the ninth day after transfer to the breeding terrarium. No amplexus was observed after egg laying and females appeared slimmer. After two more days in which no amplexus was achieved and no more spawn was deposit-

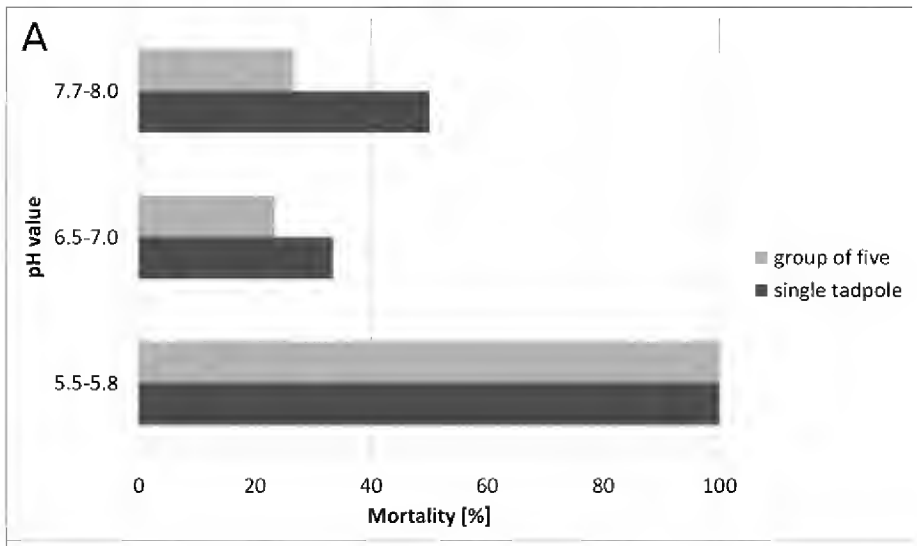

Fig. 4. (A) Mortality rate of different test groups until metamorphosis. **(B)** Average growth rate of the different test groups. **(C)** Number of tadpoles metamorphosed per day after hatching (O = osmosis water, P = pond water, number indicates individuals per container).

ed all frogs were relocated into their regular terrarium. Males continued calling infrequently, females showed no further reproductive behavior.

About three weeks after relocating the toads into their regular terrarium at least one female toad spawned again. Several egg clumps were found in the water, the spawn was attached to moss and leaves. As before, these eggs were relocated into an aquarium measuring 30 × 30 × 30 cm and raised in this tank at a temperature of 22–25 °C and a water depth of about 20 cm.

Development

Development of eggs and *M. klappenbachi* tadpoles have not been documented in the wild and development mostly correlated the records of Kurth et al. (2013) and Bland (2015). Tadpoles hatched within two to four days after spawning. There was no difference in hatching rate between eggs deposited in clumps and those deposited individually. After giving a few drops of the food mixture into water tadpoles seemed to actively seek for food on the ground. Larvae did also feed by rasping algae from the object slide. The first tadpoles metamorphosed after 19 days, whereas the last tadpoles of the test groups needed much more time to complete their development and left the water after 36 days. However, there were still a few tadpoles left in the bigger aquarium (30 × 30 × 30 cm), which did not metamorphose by this time. They were still fully aquatic and did only grow hind legs or no legs at all. After 83 days the last two of these remaining larvae metamorphosed, being the same size as the earlier metamorphed toadlets.

Just after reabsorbing the tail the small toadlets measured 6–7 mm (snout-vent-length). At this stage their coloration was dark grey to black without the conspicuous yellow markings (see Fig. 3 A). Typical patterns developed after one to two weeks (see Fig. 3 B). However, all bred toadlets did not develop any red coloration on their ventral surface, unlike the wild caught adults which showed ventral flash markings colored yellow. The first three weeks young toads were fed tropical springtails (*Collembola* sp.) once a day, so that food was always available. Afterwards they were fed every second day.

Mortality

All larvae kept in water with the lowest pH value (pH 5.5–5.8) survived for at least two days, but then died within the following four days. Tadpoles raised in water with the highest pH value (pH 7.7–8.0) had a total mortality rate of 30.56% (11 out of 36); the singly kept larvae had a mortality of 50.00% (3 out of 6). Those kept in groups of five had a mortality of 26.67% (8 out of 30). Those larvae which were kept in osmosis water with a pH value of 6.5–7.0 had a total mortality rate of 25.00% (9 out of 36). Single tadpoles had a mortality of 33.33% (2 out of 6) and larvae raised in groups of five showed a mortality of 23.33% (7 out of 30) (Fig. 4 A).

In the first eight days after hatching the total number of deceased tadpoles was at the highest level. After this period there were only occasional losses in the different test groups. In the test group of single tadpoles kept in pond water (P–1) there were no losses of larvae after day six.

Growth rate

Tadpoles in water with the lowest pH value of 5.5–5.8 did not show any sign of growth before they died. The group size did not show an influence on the growth of the tadpoles in the more alkaline pond water (P–1, single tadpole, and P–5, five tadpoles per box). In osmosis water the single-kept tadpoles (O–1) grew slower than tadpoles in groups of five larvae per box (O–5). Aside from these results, tadpoles grew faster in pond water than in

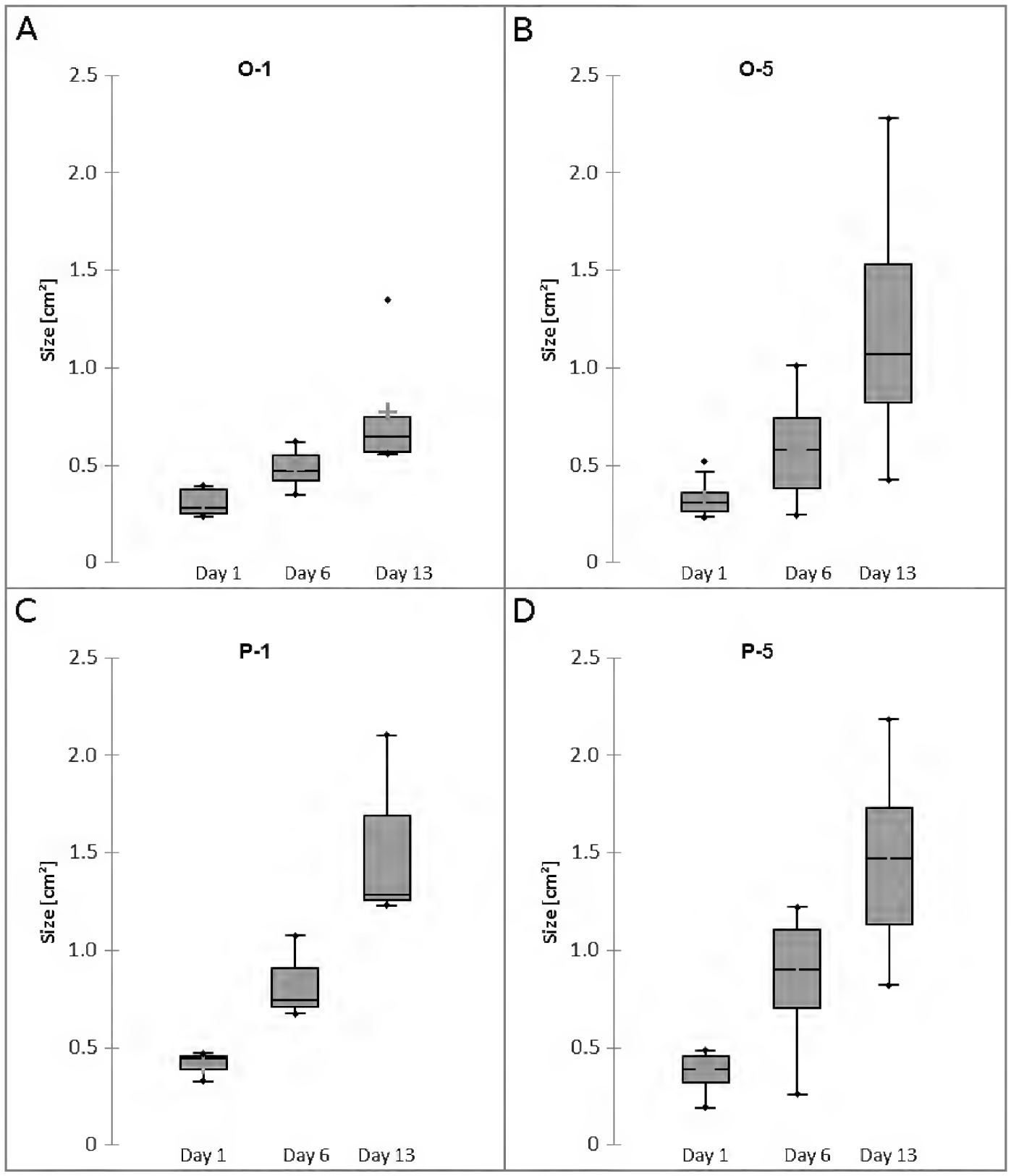

Fig. 5. Body size of different test groups. **(A)** Single tadpole, O–1, and **(B)** five tadpoles per box, O–5, in osmosis water. **(C)** Single tadpole, P–1, and **(D)** five tadpoles per box, P–5, in pond water.

osmosis water (compare Fig. 4 B).

Single kept tadpoles raised in osmosis water (O–1) showed the slowest growth of the test groups, with a mean body size of 0.773 cm² on day 13 after hatching and the biggest larva measuring 1.347 cm² (Fig. 5 A). Tadpoles in groups of five (O–5) grew faster and had a mean body size of 1.206 cm² on day 13. The largest tadpole of the test groups was in the prior group and measured 2.281 cm² on the third measurement (Fig. 5 B). The growth rate of tadpoles raised in pond water was quite similar in both test groups. On day 13, single kept larvae (P–1) had a mean body size of 1.537 cm² and a maximum size of 2.102 cm² (Fig. 5 C), while tadpoles reared in groups (P–5) showed a mean body size of 1.441 cm² with the largest tadpole measuring 2.183 cm² (Fig. 5 D).

Metamorphosis

On day 19 after hatching the first tadpoles of three test groups (P–1, P–5, and O–5) metamorphosed. The number of tadpoles metamorphosing per day reached its highest level on this day: in total seven larvae metamorphosed, four out of these seven tadpoles were in group P–5. The first tadpoles of the fourth group (O–1) metamorphosed on day 23. The last two larvae metamorphosed on day 36 (Fig. 4 C).

Discussion

Based on our husbandry experiences, the keeping, breeding, and rearing of *Melanophryniscus klappenbachi* in captivity is rather easily achieved. Most noticeable in the lifecycle of Klappenbach's Red-bellied Frog is of course the rapid larval development with the first tadpoles completing metamorphosis after 19 days at pH values between 6.5 and 7.9. This fast development represents an adaptation to the climate in their natural habitat. After heavy rainfalls adults start breeding in the emerging small temporal water bodies, which have a high desiccation risk due to drying up of these small puddles. Thus, the fact that a few tadpoles did not metamorphose after 80 days is rather surprising and has never been reported.

Table 2. Husbandry parameters for adult breeding groups of *Melanophryniscus klappenbachi* according to Kurth et al. (2013), Bland (2015), and data from this study.

	Kurth et al. (2013)	Bland (2015)	own data
Breeding group	12 adults	3 adults (2 males + 1 female)	8 adults
Terrarium size (L × W × H)	80 × 40 × 40 cm	46 × 39 × 30 cm	120 × 50 × 50 cm
Water part (L × W)	30 × 10 cm	shallow water dish	120 × 12 cm
Water depth	10 cm	—	2 cm
Temperature	—	22–26 °C	22–26 °C
Hibernation: duration; temperature	3 weeks; 8 °C	4 days; 5–8 °C (only the female)	not applied
Rainy season: duration; temperatures	—	— ; 20–25 °C	11 days; 22–26 °C
Rain chamber (L × W × H)	40 × 50 × 40 cm	60 × 45 × 45 cm	60 × 50 × 70 cm
Water part (L × W)	1/4 of the terrarium (500 cm²)	60 × 45 cm (only floating cork bark as land areas)	60 × 50 cm (only xaxim, a root and plants as land areas)
Water depth	5 cm	10 cm	6 cm
Nourishment	—	Hatchling crickets, *Drosophila* sp., *Siera* sp.; supplemented with Repashy Calcium Plus	Hatchling crickets, *Drosophila melanogaster*, *D. hydei*; supplemented with herpetal powder (Amphib, Mineral + Vitamin D3, Complete Terrarium)

It was not known that tadpoles of *M. klappenbachi* might stay in the larval stage for a longer time before metamorphosing. This behavior was only observed in the tadpoles which were kept in the bigger aquarium with a water level of at least 20 cm, whereas none of the test animals in the small boxes (10 × 10 × 10 cm) showed this long-time larval stage. A possible reason for this might be intraspecific competition amongst tadpoles. The availability of food and other resources could directly influence length of the larval stage as well. It could also be possible that tadpoles might be able to sense water levels of their surrounding environment so larvae could metamorphose before water levels decrease. Perhaps it is an adaptation, which allows a few tadpoles to survive in deeper water pools increasing overall species survivorship as a "backup." If the majority of the first, fast metamorphosing froglets die due to unstable environmental conditions, these "backup" tadpoles could increase the persistence of the species. Richter-Boix et al. (2011) investigated the influence of drying conditions on larval development in anurans and found plasticity of development across different taxa. Therefore, it would be an interesting approach for future studies on *M. klappenbachi* to investigate the influence of desiccation stress on the duration of tadpole development and growth rates.

For the last steps of metamorphosing, growing legs, and reabsorbing the tail, tadpoles did not require more time than larvae metamorphosing earlier, these steps were accomplished in only two to three days. The presence of long-term tadpoles under natural conditions might provide a steady supply of metamorphosed toads to their environment. However, this is currently not known from wild populations of *M. klappenbachi* and further field studies are suggested.

Water with a low pH value of 5.5–5.8 had a lethal effect on tadpoles within a few days and tadpoles which survived for five or six days in this water did not show any sign of growth. As *M. klappenbachi* deposits its eggs into small ephemeral ponds produced by rainwater, the pH value of these breeding sites is directly dependent on the characteristics of rainwater and soil. Therefore, the ground in Klappenbach's Red-bellied Frog's native environment has most likely neutral or even alkaline characteristics. Hence acid rain, which for example could occur due to air pollution, might be a possible future threat to tadpoles of *M. klappenbachi*. The burning of woodland is a common procedure in slash-and-burn agriculture to establish land for agricultural cultivation and can lead to a higher amount of acidity in regional rainfall (Tinker et al. 1996).

In the groups of five tadpoles it was often the case that there were three or four big tadpoles which grew faster than the remaining one or two. These remaining tadpoles showed slower growth, stayed smaller for a longer time, and metamorphosed a few days later than the larger tadpoles in the group. Additionally, these smaller specimens showed a higher mortality rate. Though live cannibalism was not observed, the deceased tadpoles were often partly or even completely eaten by their kin. Intraspecific competition for resources, mostly food and space, is a likely explanation for this observation. However in the single kept tadpoles there were a few slow-growing specimens (compare growth rate, Fig. 4 B). Since these tadpoles were not influenced by conspecifics and intra-

specific competition for food, the difference in growth might be at least partly genetically controlled. Small and weaker tadpoles might be predestined to be cannibalized by conspecifics and thus make up an important source of food, as there could be a lack of other food options in ephemeral breeding sites. A similar case is the cannibal morph of some *Ambystoma tigrinum* larvae, which have the genetic capacity to develop distinct characters adapted to feeding on conspecifics and other salamanders (Rose and Armentrout 1976).

Breeding of Klappenbach's Red-bellied Frog is often stimulated by a brumation period followed by the simulation of a rainy season (Kurth et al. 2013; Bland 2015; see also Table 2). However, our data indicates that a brumation is not a crucial factor for the successful mating and breeding of *M. klappenbachi*. After a long dry season of five to six months we relocated toads into the rain chamber (without hibernating at low temperatures) and males began to call almost immediately. This method is less stressful for the toads, thus it is recommended to use a mild brumation period as described by Bland (2015), if females do not deposit eggs after one to two weeks in the rain chamber.

Klappenbach's Red-bellied Frogs rely on the availability of very small prey items. Anything larger than a large *Drosophila* sp. was observed to not be eaten. Fruit flies, pinhead crickets, small isopods (e.g., *Trichorhina tomentosa*), and springtails are easily accepted food items, which can be purchased in pet stores and online shops, and are consumed willingly by adult toads. One interesting observation made while feeding breeding groups was that fully grown adult toads preferred smaller prey items to those larger in size. It was noticed that while feeding fruit flies and springtails at the same time springtails were favored over fruit flies.

One problem in rearing young metamorphosed toadlets was the need to have the smallest food items available. We fed these toadlets only springtails, as these are the smallest available food insects which can be purchased in most pet stores. It is most probable as well that small toads would feed on mites, tiny ground-dwelling insects, and other invertebrates. *Drosophila hydei* were fed to toadlets after two to three months though only the larger toads managed to catch these fruit flies successfully. Smaller toads tried to eat these flies, too, but showed problems swallowing them. None-the-less, larger toadlets preferred to feed on springtails.

To prevent high mortality, toadlets need to be kept on humid ground with high air humidity. For that purpose a few layers of the artificial material Hygrolon® were used and worked well keeping humidity high in containers. The Hygrolon® layers soaked up water, thus ensuring a high humidity in rearing boxes. However, with advancing age the froglets prefer dryer areas in their rearing containers and seem to suffer from ground humidity that was too high, especially in combination with insufficient air ventilation. Thus, toads must be observed carefully in the first few weeks and months to recognize the right time to decrease humidity or to relocate toadlets into a dryer box. The right humidity turned out to be a crucial factor in the husbandry of Klappenbach's Red-bellied Frogs, especially in rearing young toads.

Introducing captive breeding programs and maintaining reserve populations in captivity might be one basic requirement for adequate *ex situ* conservation arrangements. Together with natural history studies the husbandry and captive breeding of endangered species give an insight into behavior and leads to a better understanding of amphibians, the most endangered group of vertebrates. Currently, *M. klappenbachi* is listed as Least Concern by the IUCN Red List of Threatened Species due to its relatively wide distribution, large and stable populations, and its occurrence in several protected areas in both Paraguay and Argentina (Aquino et al. 2004). Nevertheless Aquino et al. (2004) have stated that more research on the species' distribution and the effects of the pet trade are necessary. Furthermore the explosive breeding behavior of this species makes it more vulnerable to diseases, as many adults gather in small vernal pools for mating and spawning. As Bland (2015) has noted, *Batrachochytrium dendrobatidis* or other infectious diseases could become a serious threat for Klappenbach's Red-bellied Frog and could lead to severe population declines or extinction. According to the IUCN Red List many other species of the genus *Melanophryniscus* are listed as Near Threatened or Threatened, with three species listed as Critically Endangered (i.e., *M. admirabilis*, *M. langonei*, and *M. peritus*) and for some species sufficient data is missing suggesting further field studies are necessary (Acquino et al. 2004). These endangered species might benefit from detailed knowledge about the husbandry and reproduction in captivity of a closely related species like *M. klappenbachi*, as the methods described herein may be applicable to them as well. This knowledge can be used to build up reserve populations and to set up breeding programs to returning captive produced specimens to the wild, if necessary.

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Rediscovery of the rare Star Mountains Worm-eating Snake, *Toxicocalamus ernstmayri* O'Shea et al., 2015 (Serpentes: Elapidae: Hydrophiinae) with the description of its coloration in life

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***Abstract.*—A series of photographs of the recently described Star Mountains Worm-eating Snake, *Toxicocalamus ernstmayri* O'Shea et al., 2015, taken at the Ok Tedi Mine in the Star Mountains, North Fly District, Western Province, Papua New Guinea, represents only the second record of this poorly-known species. *Toxicocalamus ernstmayri* was hitherto only known from its holotype, collected in December 1969 at the village of Wangbin approximately 13.2 km ESE of the photo locality. The Ok Tedi snake was observed and photographed during the day in October 2015 as it moved across a section of active mine workings, before retreating into dense montane rainforest. This series of photographs constitutes the first sighting of this snake in 45 years and the first sighting of a living animal, providing evidence of the species' continued existence in an area of considerable environmental and demographic changes brought about by human development. These images also provide evidence of its startling coloration in life.**

Keywords. Elapidae, *Toxicocalamus ernstmayri*, snake, Star Mountains, Western Province, Papua New Guinea

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The genus *Toxicocalamus* Boulenger, 1896 currently comprises fifteen taxa (fourteen species and one subspecies) of diurnal, semi-fossorial to terrestrial, secretive, vermivorous elapid snakes that are endemic to the island of New Guinea and nearby islands. Several species are poorly represented in museum collections, and the most recently described species, *Toxicocalamus ernstmayri* O'Shea et al., 2015, is one of four species known only from their holotypes, the others being *T. grandis* (Boulenger, 1914), *T. mintoni* Kraus, 2009, *T. pachysomus* Kraus, 2009, and *T. cratermontanus* Kraus 2017. The holotype of *T. ernstmayri* (Museum of Comparative Zoology, Harvard University, accession number R-145946) is also the largest specimen so far recorded for the genus, with a snout-vent length (SVL) of 1,100 mm, and a total length of 1,200 mm (O'Shea et al. 2015).

The holotype of *Toxicocalamus ernstmayri*, an adult female, was collected by one of us (FP) at Wangbin Village in the Star Mountains (5°14'26.72"S, 141°15'31.92"E, elev. 1,468 m), North Fly District, Western Province, Papua New Guinea, on 23 December 1969. The snake had been killed by a villager and handed to FP, a *kiap*[1] patrolling the area. It was originally accessioned into the museum collection as *Micropechis ikaheka* Lesson, 1830, due to its superficial resemblance to that taxon.

We here report on the second individual of *T. ernstmayri*, the first seen and photographed in life. The snake was sighted by one of us (BP) at 0750 hrs on 9 October

[1]*Kiap* is a pidgin word derived from the German word *Kapitän*, which was applied to Australian pre-independence government patrol officers. Fred Parker served as a kiap from 1960–73, being based in Western Province from 1968–73.

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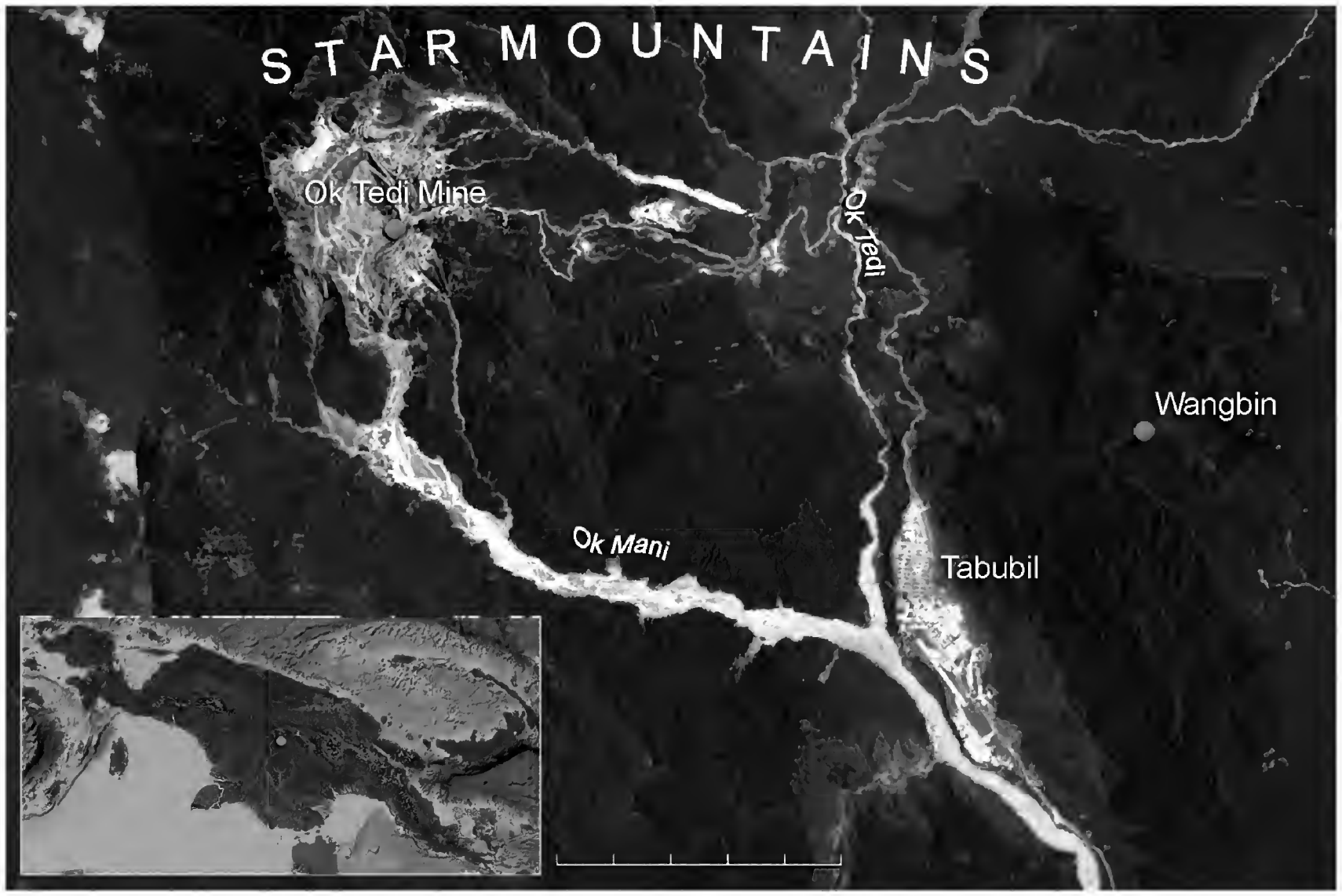

Fig. 1. Satellite map (derived from Google Earth) of the southern Star Mountains, North Fly District, Western Province, Papua New Guinea, with yellow dots on the larger map indicating two localities (Wangbin and Ok Tedi Mine), approximately 13 km apart, where *Toxicocalamus ernstmayri* has been recorded. The main town is Tabubil at the confluence of the Ok Tedi and Ok Mani, which flow into the Fly River. Scale = 5 km. The inset map illustrates the location of the larger map in relationship to the rest of New Guinea.

2015, as it crawled across an area of active mine workings along the west wall at the Ok Tedi Mine (5°12'53.77"S, 141°08'38.57"E, elev. 1,670 m) approximately 13.2 km WNW of Wangbin, in the North Fly District where the holotype was collected (Fig. 1). It was observed for approximately 20 min and photographed several times.

The snake was not captured and measured, but as it can be seen completely spanning a 747 mm tire track (Fig. 2A) its total length is certainly > 750 mm (estimated as ca. 850 mm). It was observed and photographed as it crossed open ground (Fig. 2B), rubble piles (Fig. 2C), and passed underneath a stationary digger (Fig. 2D), until it disappeared into the vegetation on the steep slope at the top left of Fig. 3.

The snake can be identified as a member of the genus *Toxicocalamus* by the presence of six supralabials and the lack of the temporolabial scale (Fig. 4B'). The only other terrestrial Papuan elapid genus to lack a temporolabial scale is *Pseudonaja*. An anterior body dorsal scale count of eight, from the vertebral scale row to the lowest dorsal scale row, can also be discerned from the images (Fig. 4C', D'), indicating an anterior dorsal scale count of 15. There does not appear to be any head scute fusion although this is harder to discern with certainty from the images. The patterning of this snake in life can be seen clearly: it has a yellow body with large grey basal spots on each dorsal scale, and a grey cap to the head. This description agrees very closely with that given by Parker (1982: 55) for the aberrant *Micropechis ikaheka*, which would become the holotype of *Toxicocalamus ernstmayri*:

> *"One snake taken at Wangbin (1500 m above sea level) in the Star Mountains differed so much in colouring from those at Kiunga and Ningerum that it may well represent another species. It was brought in already dead by a Wangbin villager. People there agreed with him that it was extremely rare in the area. The head was black, the lips bright yellow. The body scales were a deep yellow, each having a grey anterior tip. The amount of pigmentation on each scale decreased from the vertebral row towards the outermost laterals, and increased evenly along the body, with the tail darkest. There were no indications of any bars on the body. The ventral surfaces were uniform yellow."*

The characters observed in the photographs of the newly observed individual are clearly diagnostic of *T. ernstmayri* and allow us to make an unequivocal species determination. The only other genus with which this snake can be confused is *Micropechis*, which exhibits a temporolabial scale (Fig. 5). Although entirely yellow specimens of *M. ikaheka* are known, they are confined to the Vogelkop Peninsula, West Papua Province, western New Guinea; all specimens of *M. ikaheka* known from PNG are strongly banded on the posterior

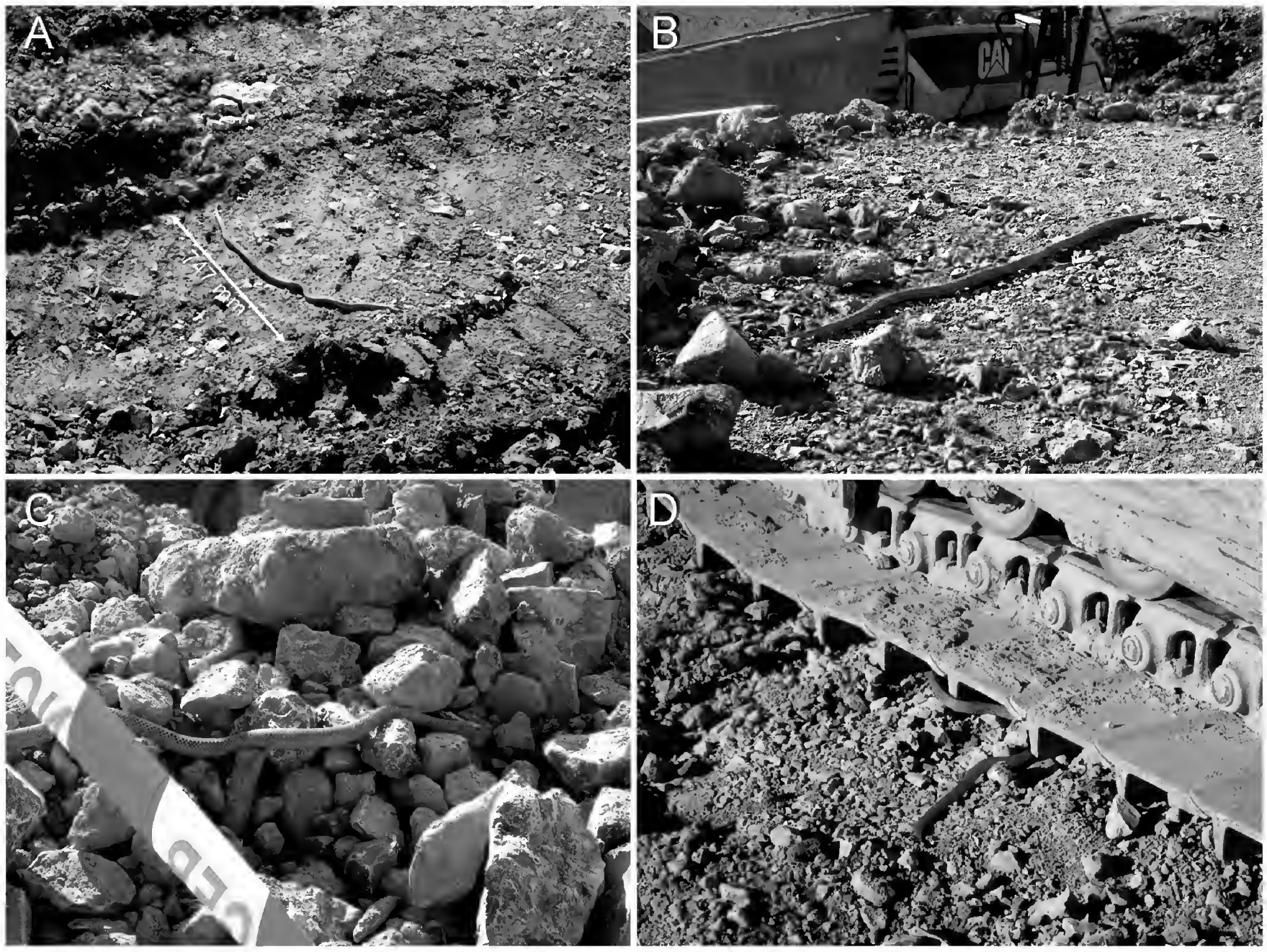

Fig. 2. The first live individual of *Toxicocalamus ernstmayri*, observed and photographed in broad daylight at the Ok Tedi Mine, North Fly District, Western Province, Papua New Guinea. **(A)** The individual's serendipitous crossing of a 747 mm wide tire track allowed an approximation of its total length as near 850 mm. **(B)** The snake moves in a straight line across open ground. **(C)** Slower movement across a rubble pile allowed a more detailed examination of head and body scales (see Fig. 4). **(D)** The individual moving under the tracks of a stationary digger. *Photos by Blaise Paivu.*

body. At an SVL > 750 mm total length this individual of *T. ernstmayri* would appear to be a subadult, as it is considerably shorter than the holotype (SVL 1,200 mm). The encounter with an unusual, "golden" snake at the Ok Tedi Mine was sufficiently noteworthy, even in Papua New Guinea where snake encounters are commonplace, that it was presented in the mine's own magazine (*Ok Tedi Weng* magazine, Issue 1, 2017, p. 6).

Topography

The source of the Ok Tedi[2] lies at approximately 2,900 m elevation in the central Star Mountains (Hyndman and Menzies 1990), just north of the provincial border between Western and Sandaun (formerly West Sepik) Provinces of PNG, and approximately 28 km east of the international border with Papua Province, Indonesian New Guinea. From its source the Upper Ok Tedi flows rapidly south through extremely rugged mountainous terrain to meet the Ok Mani, flowing in from the southern slopes of Mount Fubilan, at an elevation of 400 m, just to the west of Tabubil. The distance travelled from the source of the Ok Tedi to the Ok Mani confluence is only ca. 28.5 river kilometers (23 km in a direct line), but the river has already lost 2,500 m in elevation. The distance from Tabubil to the confluence of the Ok Tedi with the Fly River at d'Albertis Junction[3] is a further 170 river kilometers (100 km in a direct line) with a further drop in elevation to 70 m, from where the Fly meanders first southwest, then southeast to the Gulf of Papua. The town of Kiunga on the Fly River, (upstream by 45 river kilometers, 20 km in a direct line) east of d'Albertis Junction, lies at an elevation of only 20 m, yet it is approximately 375 km from the Fly delta, while the actual distance is closer to 800 river kilometers due to its meandering course across the low-lying flood plains (Halse et al. 1996).

The Ok Tedi Mine is located on the slopes of Mount Fubilan (2,084 m), "a copper mountain with a gold cap" (Knox 2013), at an elevation of approximately 1,700 m. It is approximately 12 km northwest of the nearest popu-

[2]*Ok* = river, in the local Wopkaimin language (Keig 2001), the river is therefore known as the Ok Tedi, not the Ok Tedi River. In 1876 the Italian naturalist-explorer Luigi Maria d'Albertis (1841–1901) was the first foreigner to discover and navigate the lower reaches of the Ok Tedi, which he named the Alice River (d'Albertis 1879, 1880), in honor of an acquaintance, Miss Alice Hargrave.

[3]D'Albertis originally called the confluence of the Alice River (Ok Tedi) with the Fly "Snake Junction" because he captured a python there (d'Albertis 1880) but today it is named in his honor.

Fig. 3. View of an actively worked area of the Ok Tedi Mine. The observed individual of *Toxicocalamus ernstmayri* eventually disappeared into the vegetation on the slope in the top left of the photograph. *Photo by Blaise Paivu.*

lation center, the town of Tabubil which was established to support the mine, yet the mine lies over 1.2 km higher. Tabubil, located at only 457 m elevation, is approximately 450 km from the coast. The steepness of the southern Star Mountains, rising by 1,200 m in elevation over only 12 km in horizontal distance, contrasts with the almost imperceptible south-north increase in elevation (< 500 m over 450 km) of the Trans-Fly Region as a whole.

At 1,700 m elevation, the Ok Tedi Mine is approximately 230 m higher than Wangbin Village (1,468 m elevation), the type locality of *T. ernstmayri*, suggesting that this snake is probably confined to mid-montane elevations in the Star Mountains. It is unlikely that it occurs as low as Tabubil (elevation < 500 m), given the complete lack of any specimens from there despite the large-scale development and burgeoning human population (see below). Even within its known range, this relatively large, diurnally-active snake would seem to be rare, as this region has been fairly thoroughly investigated by biologists, including by one of us (SJR), yet no specimens have been collected or reported.

The Vegetation and Climate

Ok Tedi Mine's elevation is close to the boundary between Lower Montane Rainforest (1,000–1,800 m elevation), and Low-altitude Midmontane Rainforest (1,800–2,200 m elevation), Zones 2 and 3 respectively of Hyndman and Menzies (1990). Lower Montane Rainforest comprises mixed evergreen forest with a 20–30 m tall canopy, dominated by emergent white oak (*Castanopsis acuminatissima*) at tree height of up to 40 m, whereas Low-altitude Midmontane Rainforest is dominated by moss-covered Myrtle (*Syzygium*) and Screw Palm (*Pandanus*) with a 25–30 m canopy height.

Rainfall is high in the Upper Ok Tedi-Mount Fubilan region, with as much as 10,000 mm being recorded annually at the mine (Hearn 1995), with little seasonal variation, the lowest rainfall averaging 433 mm in November, and the highest averaging 576 mm in June (Merkel 2017). The area lies in a belt known as the "midaltitude fringe high rainfall zone" (Hyndman and Menzies 1990), which experiences continual heavy rain, defined as over 50 mm per week (Brookfield and Hart 1971), although the previous figures amount to 100–140 mm of rainfall per week. Sometimes rainfall is excessive, and on at least four days a year there will be over 100 mm of rainfall over a 24-hour period, and once every 1–3 years rainfall will exceed 150 mm in a single day (McAlpine et al. 1983). The Upper Ok Tedi-Mount Fubilan region is one of the wettest places, not only on the island of New Guinea but in the world[4].

The almost constant rainfall, and accompanying heavy cloud cover, results in lowered ambient temperatures. Temperatures recorded at several sites, at different elevations from Tabubil to Mount Fubilan, are lower than those expected for central New Guinea (Hyndman and Menzies 1990). Maximum daily temperatures range from 23.0–24.7 °C, while minimums at night range from 13.8–14.6 °C (Merkel 2017). The nights above 2,200 m are even colder with lows of 6.4 °C being recorded at Finimterr (2,300 m) (Hyndman and Menzies 1990), which means temperatures fall by 1 °C with every 200 m increase in elevation. This combination of relatively cold nighttime temperatures, almost continual rain, and dense cloud cover could in part account for the diurnal activity cycle of a relatively large snake species such as *T. ernstmayri*.

Human Development

Until the late 1960s Tabubil did not exist as a settlement. Shortly after the holotype of *T. ernstmayri* was collected by FP (late 1969) a small mining camp was established besides an airstrip (O'Shea et al. 2015: Fig. 9H) by the Kennecott Copper Corporation, who were engaged in the exploratory drilling on Mount Fubilan. Wangbin was a small neighboring hamlet on the edge of Lake Wangbin (O'Shea et al. 2015: Figs. 9A–C). During 1976–1980 the Anglo-Australian mining company BHP Billiton negotiated with the Government of Papua New Guinea to establish the mining town of Tabubil and they subsequently established Ok Tedi Mining Limited to operate the gold and copper mine.

The population of the Star Mountains Tabubil "census division" increased by 201%, from 556 to 1,676, in the decade 1980–1990 (Keig 2001), directly as a result of the establishment of the Ok Tedi Mine and the development of Tabubil. Over the same period Keig (2001) reported that the population of Western Province increased from 64,623 to 74,834, which amounts to only a 15.8% popu-

[4]The annual rainfall at the Ok Tedi Mine is close to that received by the wettest places on Earth, listed as Mawsynram, Meghalaya (11,873 mm) and Cherrapunji, Meghalaya (11,430 mm), both in northeastern India (Anonymous 2017).

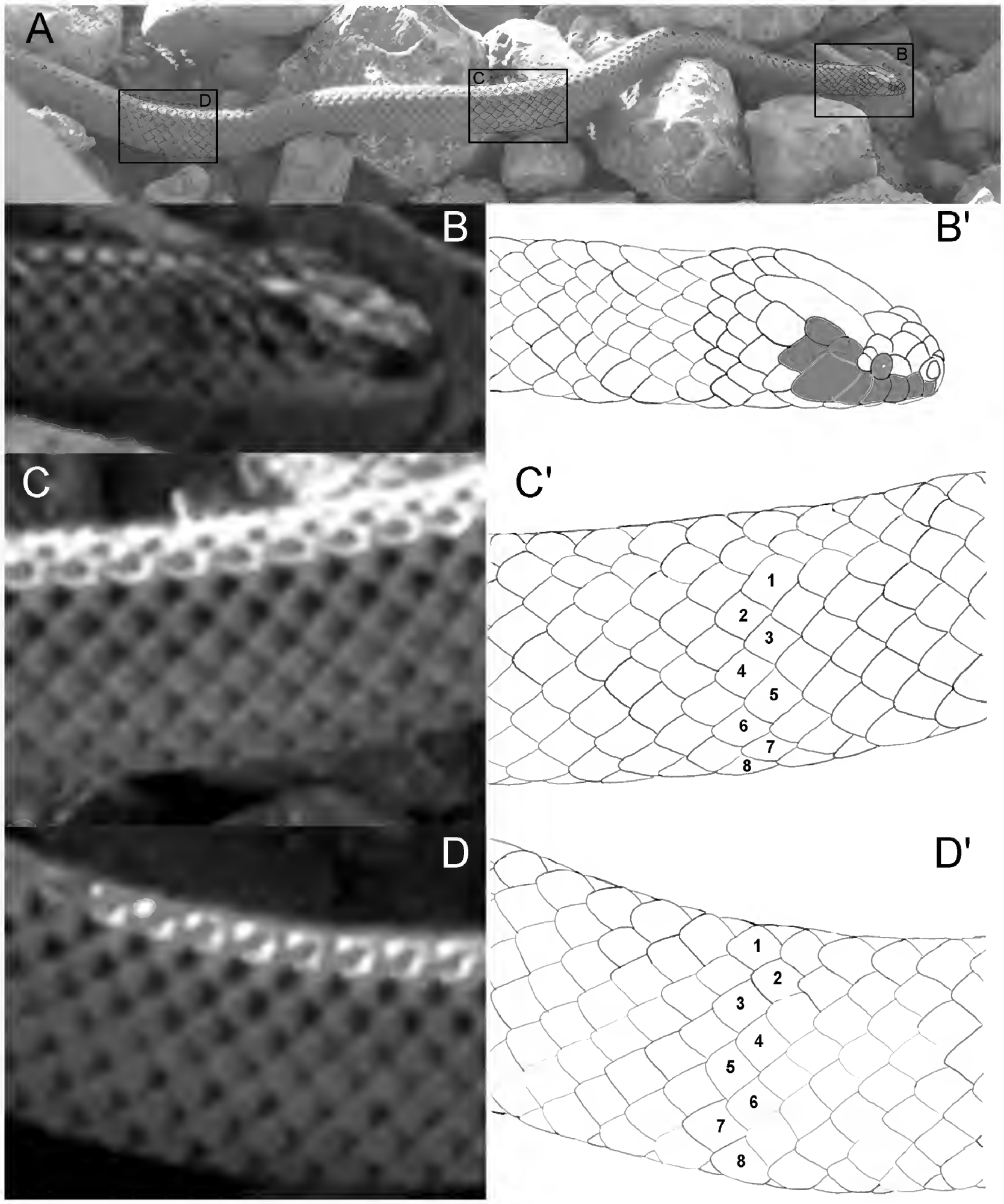

Fig. 4. Confirming the individual's identification as *Toxicocalamus ernstmayri*. **(A)** Close-up of the snake shown in Fig. 2C with insets B, C, and D indicated. **(B, B')** Head and neck in extreme close-up. Color coding of head scalation includes six supralabials (orange), one anterior temporal (yellow), and two posterior temporals (blue), but no temporolabial (see Fig. 5). The head scutes appear to comply with the colubrid-elapid nine dorsal scute arrangement (i.e., two internasals, two prefrontals, one frontal, two supraoculars, and two parietals; therefore lacking any head scute fusion, although this is difficult to discern from the magnified image with accuracy. **(C, C')** Based on the visible dorsal scales, the dorsal scale count on the anterior body is 15. The count is achieved by locating the vertebral scale row and counting down to the lowest dorsal scale row (eight scales), doubling the count, and subtracting one scale to account for the single vertebral scale row. **(D, D')** The dorsal scale count at midbody, performed as described for the previous panel, is also 15.

lation increase overall. Western Province is vast, covering 96,218 km^2 (37,150 sq mi; Blake 1972), and it is PNG's largest province (by land area), and while a report by the IUCN (1995) gave the population of the province as 110,000, with a very low overall population density of 1.14/km^2, the same report provided a population of 12,000 for Tabubil. This indicates a 716% increase in population size during the years 1990–1995, making Tabubil the largest urban population in the province, exceeding even the 8,490 population of Daru, the provincial capital in the south of the province. The 2011 census (National Statistical Office of Papua New Guinea 2014)

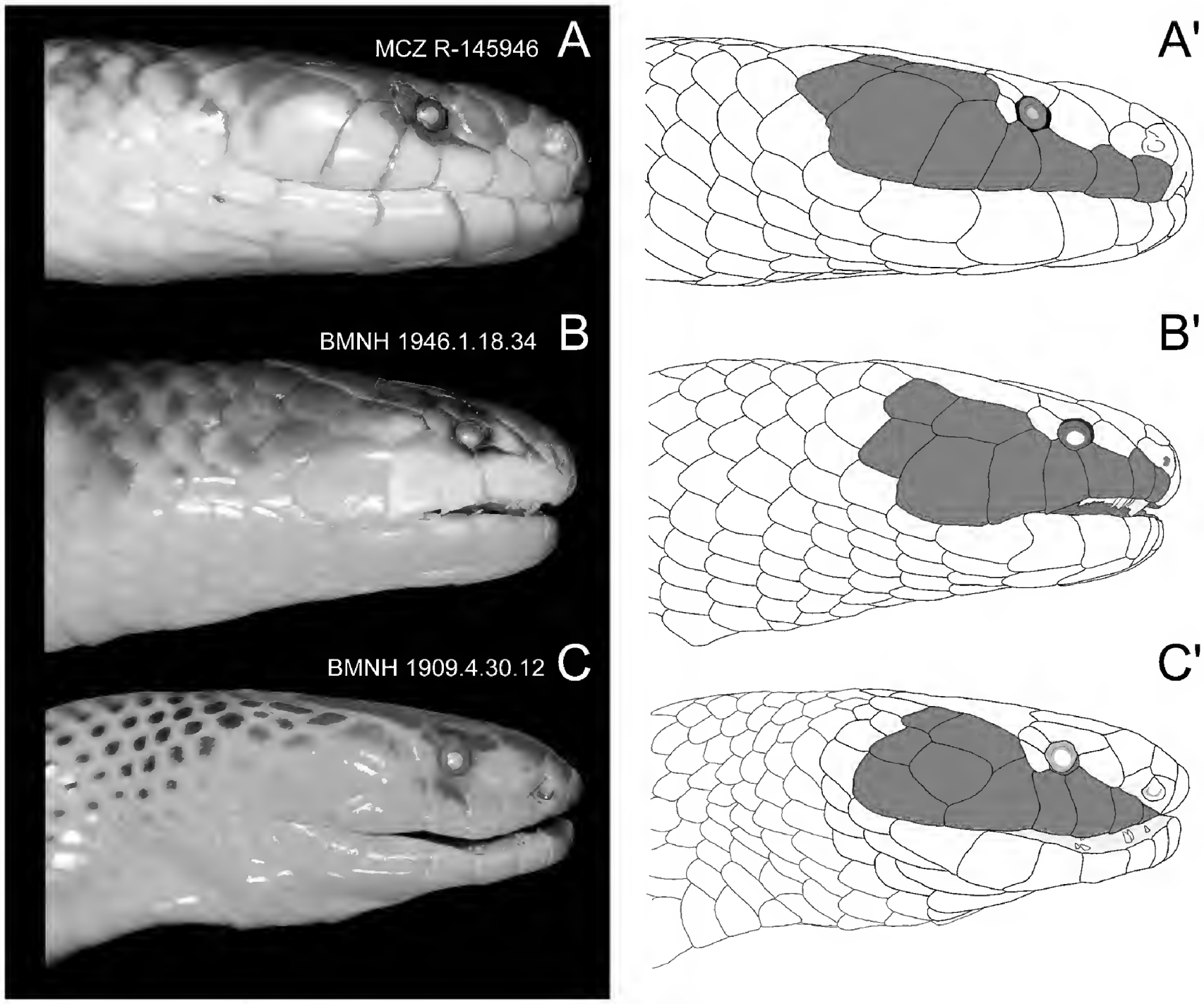

Fig. 5. Distinguishing *Toxicocalamus* from *Micropechis*. **(A, A')** Holotype of *T. ernstmayri* (MCZ R-145946) from Wangbin, Western Province, PNG. **(B, B')** Holotype of *T. grandis* (BMNH 1946.1.18.34) from Setakwa River, Papua Province, Indonesian New Guinea. **(C, C')** Yellow phase of *Micropechis ikaheka* (BMNH 1909.4.30.12) from the FakFak Peninsula, West Papua Province, Indonesian New Guinea. Color-coding of head scalation includes six supralabials (orange), a single anterior temporal (yellow), two posterior temporals (blue), and a temporolabial (red). The individual we report here clearly has the same head scute arrangement as *T. ernstmayri*.

provided a provincial population of 201,351 with 10,270 for Tabubil, 631 for Wangbin, and 15,142 for Daru, suggesting a reversal in the relative populations sizes of Tabubil and Daru. Regardless of this apparent decline the population size and development of the Tabubil area during the last 4.5 decades has been substantial. The demographics of the Tabubil population are eclectic with company employees from around the world. However, the population of the Ok Tedi Mine remains relatively small, with employees concentrated within the actual mine compound. The surrounding midmontane rainforest remains thinly populated and under-explored.

Conservation

The incursion of roads into remote rainforest areas could lead to the persecution and disappearance of vulnerable and misunderstood species like snakes. *Toxicocalamus ernstmayri* has always been an infrequently encountered species, as exemplified by Parker's (1982) comment above: "*People there agreed with him that it was extremely rare in the area.*" That it is also a diurnal species, of moderately large size, and seemingly relatively slow moving, would suggest that this species could be more vulnerable to persecution than some other taxa. It is therefore especially heartening that this snake was at no time hindered or molested as it crossed the mine workings, and that it was thought interesting and newsworthy enough to be photographed, the images then being circulated to specialists for an identification, and then finally the sighting was featured as a full-page article in the company's seven-page in-house publication, which finishes with this plea to its readers:

> *"So should you be fortunate enough to see one of these snakes in the wild, please observe it from a distance and let it go on its way. They are very rare and recorded sightings are even rarer. Like all the wild life in our foot print we should appreciate its diversity, this snake and perhaps there are other animals out there are unique to this part of PNG and the world and should be appreciated and not killed."*

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Mark O'Shea is a British herpetologist with a specialist interest in the snakes of New Guinea. He wrote *A Guide to the Snakes of Papua New Guinea* (1996) and is currently working on the second edition, expanded to encompass the entire New Guinea region, and he is also the author of four other books. Since 1986 he has made ten expeditions to New Guinea to conduct herpetological fieldwork, capture medically important elapids for snakebite research, or made films for Animal Planet or the BBC. He has worked in PNG for a variety of organizations from Operation Raleigh to Oxford University's Department of Clinical Medicine, Liverpool School of Tropical Medicine, and the Australian Venom Research Unit, University of Melbourne. O'Shea has considerable field experience in other countries in Asia, Africa, and South America, and has been engaged in fieldwork projects since the 1980s. He presented four seasons of the herpetological television series *O'Shea's Big Adventure*, for Animal Planet and Discovery Channel, and has made films with other companies and broadcasters. Mark was awarded the Millennium Award for Services to Zoology by the British Chapter of the Explorers' Club in 2000, and in 2001 was awarded an honorary Doctor of Sciences degree by his alma mater, the University of Wolverhampton, for services to herpetology. He is now Professor of Herpetology at the University of Wolverhampton and teaches the "Animal Behaviour and Wildlife Conservation and Evolution" and "Origins of Life" courses at the University. He also holds the post as Consultant Curator of Reptiles at West Midland Safari Park, in the United Kingdom. O'Shea and Kaiser (below) are the leaders of the first comprehensive survey of the herpetofauna of Timor-Leste, Asia's newest country. With ten phases of the project completed since 2009, the team has recorded upwards of 70 species, with more than twenty of these new to science. O'Shea, Kaiser, and Fred Parker (also below) are the describers of *Toxicocalamus ernstmayri*, the subject species of this paper.

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Fred Parker was born in India and migrated to Australia in 1949. While working at the Healesville Sanctuary in the late 1950s he met the herpetologist Charles Tanner and became interested in herpetology. From 1960 until 1973 he worked as a *kiap* on Bougainville, in the Central Highlands, and Western District, Papua New Guinea. Derived from the German word *kapitän*, it is the *tok pisin* name for a Government Patrol Officer, usually an Australian, in Pre-Independence Papua New Guinea. During this time Parker collected many herpetological specimens for Ernest Williams, at the Museum of Comparative Zoology (MCZ), Harvard, and Richard Zweifel, at the American Museum of Natural History (AMNH), New York. He also collected a large number of death adders for venom research and antivenom production by Tanner at the Commonwealth Serum Laboratories (CSL), Melbourne, Australia. From 1973 he worked for the Wildlife Division in Port Moresby, on projects as diverse as crocodiles and butterflies, and rose to the position of Head of the Division, before returning to Australia in 1979. He has authored and coauthored numerous papers on the herpetofauna of PNG, including the original description of *Toxicocalamus ernstmayri*. Two frogs (*Cornufer parkeri* and *Xenorhina parkerorum*), one turtle (*Chelodina parkeri*), a skink (*Tribolonotus parkeri*), and three snakes (*Bothrochilus fredparkeri*, *Gerrhopilus fredparkeri*, and *Tropidonophis parkeri*) are named in his honor.

Stephen J. Richards is an Honorary Research Associate at the South Australian Museum in Adelaide, Australia with a special interest in the herpetofauna of New Guinea. Since 1991 he has made approximately 50 expeditions to New Guinea to conduct herpetological fieldwork, and he has co-authored more than 130 publications about frogs and reptiles of that region, including the formal descriptions of nearly 100 new species discovered during these expeditions. He has published three field guides to frogs of local regions in New Guinea and the Solomon Islands. Stephen is the Regional Chair for Melanesia of the IUCN's Amphibian Specialist Group and a member of the Papua New Guinea Government's Biodiversity Expert Group. Richards has two frogs (*Hylophorbus richardsi* and *Litoria richardsi*) and a skink (*Cryptoblepharus richardsi*) named in his honor.

Hinrich Kaiser is a German-American herpetologist and educator with a research focus on biodiversity and conservation of tropical environments. A passion for scuba diving with experiences in the arctic and the tropics led Hinrich to study marine biology at McGill University and the University of Victoria in Canada. After an inspiring semester learning about amphibians and reptiles in David Green's herpetology class in the Redpath Museum, Kaiser found his true calling and earned his Ph.D. at McGill with a dissertation on the systematics and biogeography of Lesser Antillean frogs. After a Boehringer Ingelheim postdoctoral fellowship at the University of Würzburg, Germany, he spent five years as Professor of Biology at La Sierra University, Riverside, California, USA, before accepting his current position in the Department of Biology at Victor Valley College in Victorville, California, USA. Kaiser holds an appointment as Research Associate with the United States National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA. He currently serves as an Editor-in-Chief of *Herpetology Notes*, but his interests in international affairs and music also led him to memberships on the International Advisory Board of the Foundation for Post-Conflict Development, New York, and on the Advisory Council of the Baltimore Symphony Youth Orchestras. Kaiser serves as a member of the Executive Committee of the World Congress of Herpetology. His most recent publications have focused on the herpetofauna of Timor-Leste and nearby areas of Wallacea, as well as on the defense of herpetological taxonomy against taxonomic vandalism. He was also a coauthor on the original description of *Toxicocalamus ernstmayri*. His educational specialty is to expose community college students to biological, cultural, and historical experiences overseas, including canopy walks in Brunei, cooking classes in Bali, tracking Komodo dragons on Rinca Island, homestays in Cuba, and surveying Pacific atolls.

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Amphibians and reptiles of Parsa National Park, Nepal

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***Abstract.*—We report the results of a herpetofaunal inventory between July, 2014 and March, 2017 of Parsa National Park that detected 51 herpetofaunal species. Three amphibians (*Microhyla nilphamariensis*, *Sphaerotheca breviceps*, and *Uperodon taprobanicus*), two Gecko species (*Hemidactylus flaviviridis* and *H. frenatus*), one Agamid (*Sitana fusca*), two Skinks (*Eutropis carinata* and *Sphenomorphus maculatus*), 13 snakes (*Ahaetulla nasuta*, *Bungarus lividus*, *Coelognathus helena*, *Coelognathus radiatus*, *Chrysopelea ornata*, *Dendrelaphis tristis*, *Lycodon aulicus*, *Lycodon jara*, *Oligodon arnensis*, *Psammodynastes pulverulentus*, *Ptyas mucosa*, *Rhabdophis subminiatus*, and *Trimeresurus albolabris*), and one crocodile (*Crocodylus palustris*) are new records to Parsa National Park. This paper aims to highlight the understanding of amphibians and reptiles of Parsa National Park and will be a reference for herpetofaunal management in the park.**

Keywords. Herpetofauna, biodiversity, conservation, protected area, Terai-Arc Landscape, new records

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Introduction

Globally, amphibians and reptiles are among the least studied vertebrate taxa (Fazey et al. 2005). The amphibians and reptiles of Nepal have a wide range of both vertical and horizontal distribution. However, the field of herpetology has always received less priority than other vertebrates (Bhattarai et al. 2017). Among herpetofaunal species, only the gharial (*Gavialis gangeticus*) subjected to long term monitoring and conservation efforts (Acharya et al. 2017). Information on species richness and distribution of amphibians and reptiles in management plans of many Protected Areas of Nepal including Parsa National Park (PNP) are poorly documented. Past studies by Schleich and Kästle (2002) and Shah and Tiwari (2004) recorded 37 species from the PNP and lack detailed locality information. Since then, several taxonomic revisions of the species have been done. In addition to this, Kästle et al. (2013) listed eight species of herpetofauna which underestimates the species richness of the PNP. Here, we provide the comprehensive checklist on species richness with natural history data to highlight understanding of the amphibian and reptile fauna of Parsa National Park.

Study Area

Parsa National Park (PNP), the youngest National Park in the country, was established in 1984 as Wildlife Reserve and upgraded to National Park in 2017. It is geographically located within 27°15' to 27°33'N, 84°41' to 84°58'E. The unique sub-tropical dry ecosystem was established to protect habitat mainly for the resident population of wild Asian elephant (*Elephas maximus*). However, it also provides a habitat for migratory wildlife species and a dispersal site for spill-over population of Chitwan National Park to which it is connected at its western boundary and Valmaki Tiger Reserve of India to the South. Examples are the Asian one-horned Rhinoceros (*Rhinoceros unicornis*), Royal Bengal tiger (*Panthera tigris*) and Gaur (*Bos gaurus*). Understanding the potential to conserve many charismatic species, the Government of Nepal extended the area of the PNP in 2015 and the current area is 627 km^2 (Fig. 1). Besides its biodiversity conservation value, the PNP is also serving the vital needs of the large human population living south of the park by conserving water sources in the Siwalik hill and has reduced the soil erosion in the hill. The PNP includes mainly sub-tropical forests of the Siwalik and

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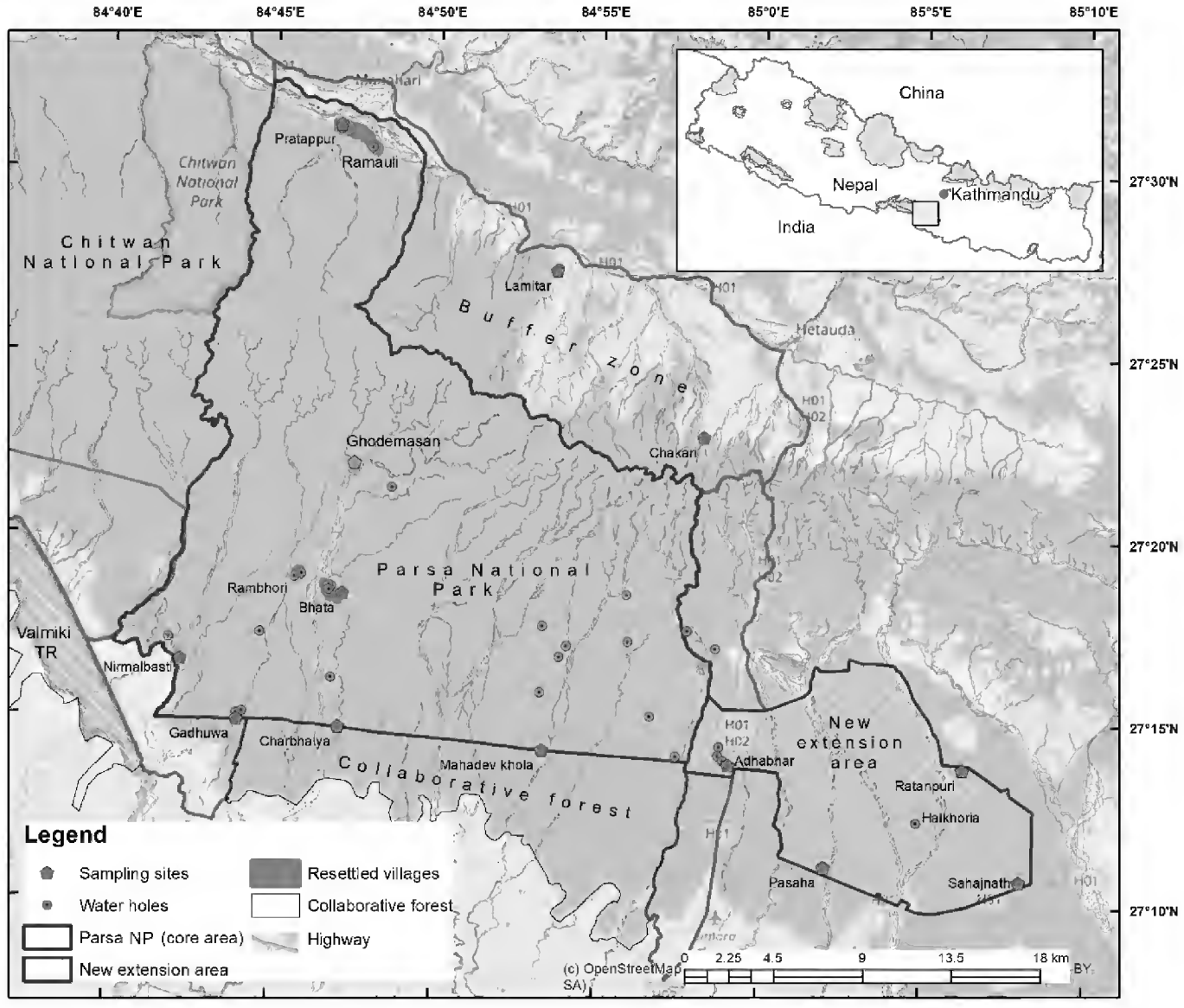

Fig. 1. Study location, Parsa National Park.

Bhabar physiographic regions of Parsa, Makwanpur and Bara districts. The vegetation is mainly dominated by Sal (*Shorea robusta*) forest, and riverbeds and flood plains are covered by *Saccharum spontaneum* and *Imperata cylindrica* (Chhetri 2003). Although the PNP is connected with Chitwan National Park (Nepal) and Valmiki Tiger Reserve (India), very little information on species richness and diversity is available (Lamichhane et al. 2017). We concentrated our search effort near permanent water bodies and artificially created ponds inside the park. Field investigations were conducted at Rambhori-Bhata, Halkhoria Daha, Amlekhganj-Hattisar, Adhabhar, Ghodemasan, Mahadev Khola, Gaduwa-line, and Nirmalbasti, Ramauli-Pratappur.

Field Methods

We conducted surveys in both the dry and wet seasons. We used the visual encounter survey protocol (Heyer et al. 1994) and active searches from 10–20 July, 2014, 15–27 March, 2015, 18–21 June, 2015, 04–10 February, 2016, 17–25 July, 2016, and 03–09 March, 2017. We covered all major sites within the park. Our search effort focused on recording the diverse herpetological community as efficiently as possible. On each expedition, we spent three hours of intensive search combined with opportunistic records. During the survey, on detection of an animal, we recorded the location, date, time, and microhabitat. We did not use dogs or chemicals or any auditory cues for species detection. However, we included opportunistic records of various herpetofauna encountered elsewhere within the PNP in our results. Photographs of detected animals were taken whenever possible and used as visual evidence for verifying species identifications. We used keys described in Smith (1935), Schleich and Kästle (2002), and Shah and Tiwari (2004) for identification. We followed Frost (2017) for nomenclature of amphibians and Uetz et al. (2017) for reptiles.

Results

We recorded 12 species of amphibians in eight genera and four families of anurans (Table 1), and 39 species of reptiles which consisted of five species of skinks, three species of Geckonids, two species of Agamids, two species of monitor lizards, 25 snake species, and one tortoise and crocodile each (Table 1). We recorded 22 additional species in the area which accounted for 51 species of the herpetofauna in the PNP. These additional species consist of three species of anurans, two species of gecko, two species of skinks, 13 snake species, and one crocodile species.

Species Accounts

AMPHIBIANS

Bufonidae (Gray 1825)

Duttaphrynus melanostictus (Schneider 1799): Recorded from Amlekhganj-Hattisar, Adhabhar, Rambhori-Bhata, Halkhoria Daha, Nirmalbasti, and Ramauli-Pratapur. This was commonly seen in and around human settlements during monsoon. Road-killed individuals of this

Table 1. Amphibians and Reptiles of Parsa National Park, Nepal. An asterisk (*) denotes new records to the area.

S.N.	Species	IUCN Status
AMPHIBIANS		
Bufonidae Gray, 1825		
1.	*Duttaphrynus melanostictus* (Schneider 1799)	LC
2.	*D. stomaticus* (Lütken 1864)	LC
Dicroglossidae Anderson, 1871		
3.	*Euphlyctis cyanophlyctis* (Schneider 1799)	LC
4.	*Fejarvarya syhadrensis* (Annandale 1919)	LC
5.	*Fejarvarya teraiensis* (Dubois 1984)	LC
6.	*Hoplobatrachus crassus* (Jerdon 1853)	LC
7.	*Hoplobatrachus tigerinus* (Daudin 1802)	LC
8.	**Sphaerotheca breviceps* (Schneider 1799)	LC
Microhylidae Gunther, 1858		
9.	**Microhyla* cf. *nilphamariensis* (Howlader, Nair, Gopalan, and Merila 2015)	LC
10.	*Uperodon globulosus* (Günther 1864)	LC
11.	**Uperodon taprobanicus* (Parker 1934)	LC
Rhacophoridae Hoffman, 1932 (1858)		
12.	*Polypedates maculatus* (Gray 1830)	LC
REPTILES		
Gekkonidae Gray, 1825		
13.	*Hemidactylus* cf. *brookii* Gray, 1845	NA
14.	**Hemidactylus flaviviridis* Rüppell, 1835	LC
15.	**Hemidactylus frenatus* Dúmeril and Bibron, 1836	LC
Agamidae Gray, 1827		
16.	*Calotes versicolor* (Daudin 1802)	NA
17.	**Sitana fusca* Schleich and Kästle, 1998	NA
Scincidae Gray, 1825		
18.	**Eutropis carinata* (Schneider 1801)	LC
19.	*Eutropis dissimilis* (Hallowell 1857)	NA
20.	*Eutropis macularia* (Blyth 1853)	NA
21.	*Lygosoma punctata* (Gmelin 1799)	NA
22.	**Sphenomorphus maculatus* (Blyth 1853)	NA
Varanidae Merrem, 1820		
23.	*Varanus bengalensis* (Daudin 1802)	LC
24.	*Varanus flavescens* (Hardwicke and Gray 1827)	NA
Typhlopidae Merrem, 1820		
25.	*Indotyphlops braminus* (Daudin 1803)	NA
Boidae Gray, 1825		
26.	*Eryx conicus* (Schneider 1801)	NA
Pythonidae Fitzinger, 1826		
27.	*Python bivittatus* Kuhl, 1820	VU
Colubridae Oppel, 1811		
28.	**Ahaetulla nasuta* (Bonnaterre 1790)	NA
29.	*Boiga trigonata* (Schneider 1802)	LC
30.	**Coelognathus helenam* (Daudin 1803)	NA
31.	**Coelognathus radiatus* (Boie 1827)	LC
32.	**Chrysopelea ornata* (Shaw 1802)	NA
33.	**Dendrelaphis tristis* (Daudin 1803)	NA

Table 1. Amphibians and Reptiles of Parsa National Park, Nepal. An asterisk (*) denotes new records to the area.

S.N.	Species	IUCN Status
Colubridae Oppel, 1811		
34.	**Lycodon aulicus* (Linnaeus 1758)	NA
35.	**Lycodon jara* (Shaw 1802)	LC
36.	**Oligodon arnensis* (Shaw 1802)	NA
37.	**Psammodynastes pulverulentus* (Boie 1827)	NA
38.	**Ptyas mucosa* (Linnaeus 1758)	NA
39.	*Sibynophis sagittarius* (Cantor 1839)	NA
Elapidae F. Boie, 1827		
40.	*Bungarus caeruleus* (Schneider 1801)	NA
41.	*Bungarus fasciatus* (Schneider 1801)	LC
42.	**Bungarus lividus* Cantor, 1839	NA
43.	*Naja naja* (Linnaeus 1758)	NA
44.	*Ophiophagus hannah* (Cantor 1836)	VU
Natricidae Bonaparte, 1838		
45.	*Amphiesma stolatum* (Linnaeus 1758)	NA
46.	*Xenochropis piscator* (Schneider 1799)	NA
47.	**Rhabdophis subminiatus* (Schlegel 1837)	NA
Viperidae Oppel, 1811		
48.	*Daboia russelii* (Shaw and Nodder 1797)	LC
49.	**Trimeresurus albolabris* Gray, 1842	NA
Testudinidae Batsch, 1788		
50.	*Indotestudo elongata* (Blyth 1854)	EN
Crocodylidae Cuvier, 1806		
51.	**Crocodylus palustris* Lesson, 1831	VU

species were frequently observed in the east-west national highway between Amlekhgunj and Adhabhar segment. This is the most common bufonid in Terai, Nepal (Fig. 2).

Fig. 2. *Duttaphrynus melanostictus. Photograph by Kapil Pokharel/NTNC-BCC.*

Duttaphrynus stomaticus (Lütken 1864): This was frequently encountered at NTNC-Parsa Conservation Program Office complex, Hattisar, Amlekhganj, Adhabhar, Ramauli-Pratappur, Bhata, and Nirmalbasti (Fig. 3). The individuals can be distinguished from *D. melanostictus* by absence of canthal black ridge and smaller tympanum.

Fig. 3. *Duttaphrynus stomaticus. Photograph by Santosh Bhattarai.*

Dicroglossidae (Anderson 1871)

Euphlyctis cyanophlyctis (Schneider 1799): The most common frog of Terai Nepal within and outside protected areas commonly encountered in water pools (Fig. 4).

Fig. 4. *Euphlyctis cyanophlyctis. Photograph by Santosh Bhattarai.*

Fejarvarya syhadrensis (Annandale 1919): The individuals we recorded had no mid dorsal line with reddish orange patches which is characteristic of this species (Schleich and Kästle 2002). We recorded this species along marshy lands in the ponds inside the park.

Fejervarya teraiensis (Dubois 1984): The calling males were recorded at puddles in Amlekhgunj, Adhabar, and Bhata. The individuals had a cream colored mid dorsal line with dorsolateral fold. According to Schleich and Kästle (2002), this species is well distributed in the entire Terai from 71 to 400 m.

Hoplobatrachus crassus (Jerdon 1853): We found an individual of this species at an army post in Gaduwaline inside the park. Shah and Tiwari (2004) also recorded this species from Parsa.

Hoplobatrachus tigerinus (Daudin 1802): This is the largest frog of Terai region. Yellow colored breeding males were frequently observed in puddles during monsoon (Fig. 5).

Fig. 5. *Hoplobatrachus tigerinus. Photograph by Santosh Bhattarai.*

Sphaerotheca breviceps (Schneider 1799): Almost toad-like, stocky with distinct supratympanal fold. We found some specimens in Halkhoria Daha and Amlekhgunj-Hattisar area during June and July and calling males were also observed. This is the first record to Parsa National Park.

Fig. 6. *Sphaerotheca breviceps. Photograph by Santosh Bhattarai.*

Microhylidae (Günther 1843, 1858)

Microhyla cf. *nilphamariensis* (Howlader, Nair, Gopalan, and Merila 2015): The type locality of this frog is Koya Golahut, Saidpur, Nilphamari, Bangladesh. Recently, Khatiwada et al. (2017) recorded it from central and eastern Nepal and proposed the Chitwan population to be *M. nilphamararіensis* based on molecular and call records. We believe the Parsa population to be *M. nilphamariensis* (Fig. 7). However, only detailed molecular study will resolve its taxonomy.

Fig. 7. *Microhyla* cf. *nilphamariensis. Photograph by Santosh Bhattarai.*

Uperodon globulosus (Günther 1864): This bulky globular frog is frequently seen during monsoon, when calling males were seen during the night in Bhata area. Shah and Tiwari (2004) also reported the occurrence of this species from Parsa National Park (Fig. 8).

Fig. 8. *Uperodon globulosus. Photograph by Santosh Bhattarai.*

Uperodon taprobanicus (Parker 1934): This frog is grayish black, and individuals have reddish-orange dorsolateral irregular bands. Individuals with a mid-dorsal line from snout to vent and with mid-dorsal line were recorded (Fig. 9). Males have folded black vocal sacs and were observed in amplexus. According to Schleich and Kästle (2002), this species is distributed from central to eastern Nepal between 100 and 300 m elevation. Bhattarai et al. (2017a) also recorded this species from Beeshazar and associated lakes, a Ramsar site.

Fig. 9. *Uperodon taprobanicus. Photograph by Santosh Bhattarai.*

Rhacophoridae (Hoffman 1932)

Polypedates maculatus (Gray 1830): Calling males were frequently observed at NTNC-Parsa Conservation Program office complex during the monsoon. This species was frequently observed on the office window and in the bathroom (Fig. 10).

Fig. 10. *Polypedates maculatus. Photograph by Santosh Bhattarai.*

REPTILES

Gekkonidae (Gray 1825)

Hemidactylus cf. *brookii* (Gray 1845): Individuals with strongly keeled dorsal tubercles and tails with spines were recorded. Schleich and Kästle (2002) recorded *H. brookii* on buildings in Chitwan National Park. However, we recorded them in dead logs inside the park in Parsa National Park (Fig. 11). This species is regarded as a species complex and has been proposed for detailed molecular studies to solve taxonomy of Nepalese populations (Rösler and Glaw 2010; Kathriner et al. 2014).

Fig. 11. *Hemidactylus brookii. Photograph by Santosh Bhattarai.*

Hemidactylus flaviviridis (Rüppell 1835): This is a common house gecko in the study area. Frequently seen at houses, park guard posts and army posts, and the temple inside the park, as well as villages nearby the park. This is the first record from the Parsa National Park.

Hemidactylus frenatus (Dúmeril and Bibron 1836): We reported two individuals of this species, photographed at Bhata-Hattisar and Gaduwa. This is the first record of this species from Parsa National Park.

Agamidae (Gray 1827)

Calotes versicolor (Daudin 1802): This is the most common diurnal agamid distributed from below 100 m to 3,200 m in Nepal (Schleich and Kästle 2002). The species was frequently observed in and out of the park boundary (Fig. 12).

Fig. 12. *Calotes versicolor. Photograph by Santosh Bhattarai.*

Sitana fusca (Schleich and Kästle 1998): This species was described from Bardibas, Mahottari district, Nepal ca. 100 km east of Parsa National Park. This is the first record of *Sitana* from Parsa National Park. This species was frequently observed at NTNC-Parsa Conservation Program office complex, Bhedaha Khola, and Darau Khola. In June 2016, a gravid female was observed nesting in the office complex, and two hatchlings of same species were encountered in August 2016 (Fig. 13).

Fig. 13. Nesting female of *Sitana fusca. Photograph by Santosh Bhattarai.*

Scincidae (Gray 1825)

Eutropis carinata (Schneider 1801): Commonly observed inside the park basking in open grassland and on rocky substrates. Observed at Kamini Daha, Bhata, Mahadev Khola, Halkhoria Daha, Ghode Masan, Ramauli-Pratappur, Sikaribasb Bhedaha Khola, and Darau Khola. This is one of the most commonly observed skinks in Nepal. However, earlier researchers did not report it from Parsa National Park (Fig. 14).

Fig. 14. *Eutropis carinata. Photograph by Kapil Pokharel/ NTNC-BCC.*

Eutropis dissimilis (Hallowell 1857): Recorded from Amlekhgunj-Hattisar, Sikaribaas basking during winter. This species is rarely seen compared to its congenerics in Parsa National Park (Fig. 15).

Fig. 15. *Eutropis dissimillis. Photograph by Kapil Pokharel/ NTNC-BCC.*

Eutropis macularia (Blyth 1853): Observed from Kamini Daha, Amlekhgunj-Hattisar, Bhata, Nirmalbasti, Ramauli Pratappur, Mahadev Khola, and Ghode Masan (Fig. 16).

Fig. 16. *Eutropis macularia. Photograph by Binod Darai/ NTNC-BCC.*

Lygosoma punctata (Gmelin 1799): Observed from Bhata, Adhabhar, Sikaribaas, and Shitalpur (Fig. 17).

Fig. 17. *Lygosoma punctata. Photograph by Binod Darai/ NTNC-BCC.*

Sphenomorphus maculatus (Blyth 1853): This species was frequently observed in the foothills of Siwaliks inside the park and found basking on the rocks of dry river beds (Fig. 18). This is the first record for Parsa National Park.

Fig. 18. *Sphenomorphus maculatus. Photograph by Santosh Bhattarai.*

Varanidae (Merrem 1820)

Varanus bengalensis (Daudin 1802): Individuals were observed at Kamini Daha, Masine area, Bhata, Adhabhar-PNP office, Bhedaha Khola, Shitalpur, and Ramauli-Pratapur. They were frequently observed at human habitations at Amlekhgunj, and one adult was rescued from the Nepal Oil Corporation's office complex. The species is frequently seen in holes of the Sal (*Shorea robusta*) trees lying on the ground and on standing trees (Fig. 19).

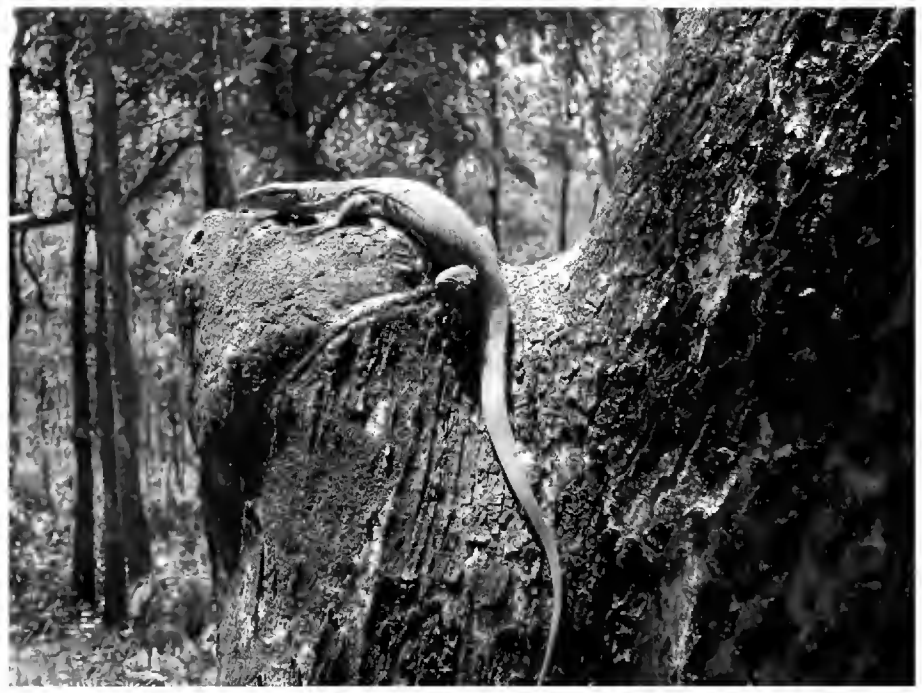

Fig. 19. *Varanus bengalensis. Photograph by Kapil Pokharel/ NTNC-BCC.*

Varanus flavescens (Hardwicke and Gray 1827): This species was frequently encountered in the buffer zone around the PNP and in agricultural lands outside the park boundary. It is a legally protected varanid of Nepal which has been accorded the highest degree of protection in Schedule-I under the National Parks and Wildlife Conservation Act, 1973. The species is facing severe threat due to illegal hunting for its flesh and skin. The skin of varanids is used for making musical instruments by local communities.

Typhlopidae (Merrem 1820)

Indotyphlops braminus (Daudin, 1803): The species was observed from Kamini Daha living inside leaf litter.

Boidae (Gray 1825)

Eryx conicus (Schneider 1801): This species was encountered at Amlekhgunj-Hattisar (Fig. 20).

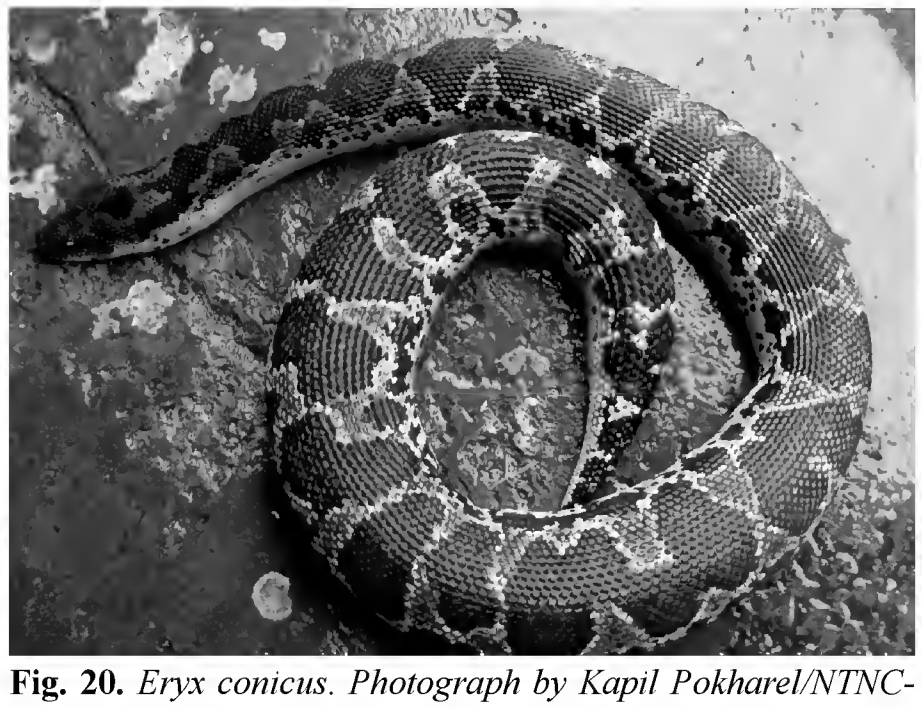

Fig. 20. *Eryx conicus. Photograph by Kapil Pokharel/NTNC-BCC.*

Pythonidae (Fitzinger 1826)

Python bivittatus (Kuhl 1820): The python is the largest snake species in Nepal and it is distributed from Nepalese Terai up to 2,800 m elevation in Nepal (Bhattarai et al. 2017). In the PNP, the species was observed from Bhata, Amlekhgunj-Hattisar, Halkhoria Daha, and Ramauli Pratapur (Fig. 21). The PNP has dry sub-tropical habitat and gets incidental fire. One injured python was found with wounds inside the park at Kamini Daha.

Fig. 21. *Python bivittatus. Photograph by Om P. Chaudhary/ NTNC-BCC.*

Colubridae (Oppel 1811)

Ahaetulla nasuta (Bonnaterre 1790): An individual of this species was observed at Mahadev Khola basking on grasses and flew to the bush when approached. Another individual was observed at Shitalpur on a *Mallotus philippensis* tree approximately 3.5 m from ground level. We report this species for the first time from the park.

Boiga trigonata (Schneider 1802): Many killed specimens were found in the buffer villages and highway between Amlekhgunj and Pathlaiya section of the National Park (Fig. 22).

Coelognathus helena (Daudin 1803): Observed from Amlekhgunj-Hattisar, Adhabhar-PNP office complex, and Ramauli Pratapur. This is the first record from Parsa National Park.

Coelognathus radiatus (Boie 1827): Dead specimens were found near human habitation, and an individual was recorded at Kamini Daha. In May and June, the species is frequently observed in buffer villages of the park, and people kill the snakes when they encounter them.

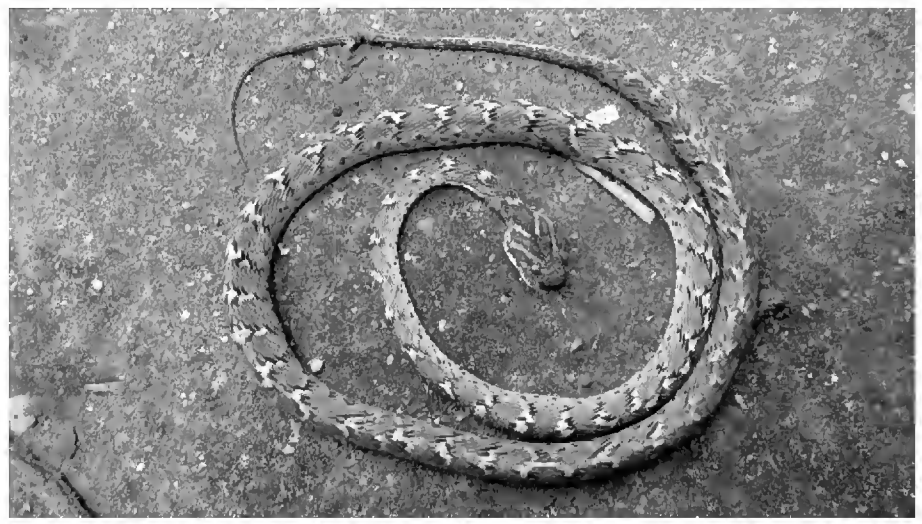

Fig. 22. *Boiga trigonata. Photograph by Kapil Pokharel/ NTNC-BCC.*

Chrysopelea ornata (Shaw 1802): A juvenile individual was observed at Shikaribas Khola, and a dead specimen was found at Amlekhgunj-Hattisar (Fig. 23). This is the first record from Parsa National Park.

Fig. 23. *Chrysopelea ornata. Photograph by Kapil Pokharel/ NTNC-BCC.*

Dendrelaphis tristis (Daudin 1803): The basking individuals were encountered at Amlekhgunj-Hattisar, Bhata-Hattisar, and Ghodemasan (Fig. 24). This is the first record from Parsa National Park.

Fig. 24. *Dendrelaphis tristis. Photograph by Om P. Chaudhary/ NTNC-BCC.*

Lycodon aulicus (Linnaeus 1758): Observed at NTNC-Parsa Conservation Program Office complex, and dead individuals were found at Amlekhgunj-Hattisar. A basking individual was frequently observed in a crevice of a cemented water tank (Fig. 25). This is the first record from Parsa National Park.

Fig. 25. *Lycodon aulicus. Photograph by Santosh Bhattarai.*

Lycodon jara (Shaw 1802): Observed at Amlekhgunj-Hattisar. According to Schleich and Kästle (2002), it is a rarely found species from Terai Nepal. However, there are published reports of it in bordering states of India as well. This is the first record from Parsa National Park (Fig. 26).

Fig. 26. *Lycodon jara. Photograph by Santosh Bhattarai.*

Oligodon arnensis (Shaw 1802): Observed from Amlekhgunj-Hattisar and NTNC-Parsa Conservation Office Complex (Fig. 27). This species is also frequently observed in Chitwan National Park.

Fig. 27. *Oligodon arnensis.* P*hotograph by Kapil Pokharel/ NTNC-BCC.*

Psammodynastes pulverulentus (Boie 1827): According to Schleich and Kästle (2002), the records of the species were from Butwal, western Nepal, and Khotang, Udaypur, and Ilam from eastern Nepal. Recently, Bhattarai et al. (2017) reported it from Ratomate-Harda Khola, Chitwan National Park. Later the species was also observed at Triveni area of Chitwan National Park. In the PNP, the species was observed at Ghodemasan area, being the first record from the PNP (Fig. 28).

Fig. 28. *Psammodynastes pulverulentus. Photograph by Tirtha Lama/NTNC-BCC*, photograph taken at Triveni, Chitwan National Park.

Ptyas mucosa (Linnaeus 1758): Animals in combat were observed on 7 June, 2016. A road-killed specimen in the segment between Amlekhgunj and Adhabhar was recorded. Individuals were frequently observed at NTNC-Parsa Conservation Office complex (Fig. 29). This report is the first record for Parsa National Park.

Fig. 29. *Ptyas mucosa. Photograph by Santosh Bhattarai.*

Sibynophis sagittarius (Cantor 1839): A specimen was found at Ghodemasan area basking on a riverbed (Fig. 30).

Fig. 30. *Sibynophis sagittarius. Photograph by Kapil Pokharel.*

Elapidae (F. Boie 1827)

Bungarus caeruleus (Schneider 1801): Specimens observed at Amlekhgunj-Hattisar. Killed specimens were found near human habitation (Fig. 31).

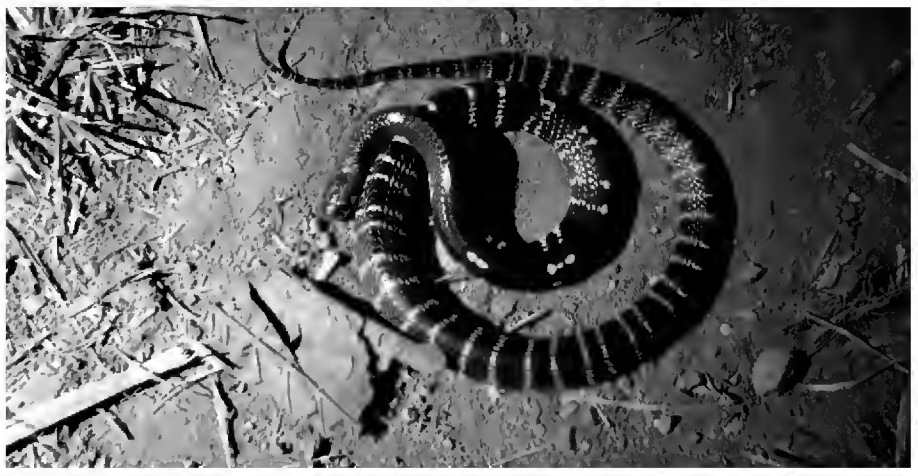

Fig. 31. *Bungarus caeruleus. Photograph by Kapil Pokharel/ NTNC-BCC.*

Bungarus fasciatus (Schneider 1801): One individual was found crawling inside Amlekhgunj-Hattisar in July 2016.

Bungarus lividus (Cantor 1839): An individual was observed at Bhata-Hattisar on forest trail towards Bhata-temple. The second individual was found killed in Amlekhgunj. This is the first record from Parsa National Park.

Naja naja (Linnaeus 1758): An individual was found basking in the riverbed of Bhedah Khola. Two individuals were found killed at human habitation at Amlekhgunj (Fig. 32).

Fig. 32. *Naja naja. Photograph by Kapil Pokharel/NTNC-BCC.*

Ophiophagus hannah (Cantor 1836): A dead specimen was recorded at Amlekhgunj-Hattisar. Another individual was observed at Shitalpur camp in November 2016. (Fig. 33).

Fig. 33. *Ophiophagus hannah. Photograph by Kapil Pokharel/ NTNC-BCC.*

Natricidae (Bonaparte 1838)

Amphiesma stolatum (Linnaeus 1758): Frequently observed at Amlekhgunj-Hattisar, Bhata-Hattisar, and Adhabhar-PNP office complex. An individual was observed feeding on *Duttaphrynus melanostictus* at NTNC-Parsa Conservation Office complex. Road kills observed in the segment between Amlekhgunj and Adhabhar (Fig. 34).

Fig. 34. *Amphiesma stolatum. Photograph by Kapil Pokharel/ NTNC-BCC.*

Xenochrophis piscator (Schneider 1799): The species was frequently observed in human habitation and a specimen was seen in the Bhata wetland (Fig. 35).

Fig. 35. *Xenochropis piscator. Photograph by Kapil Pokharel/ NTNC-BCC.*

Rhabdophis subminiatus (Schlegel 1837): Record of this species was previously not reported from the PNP. Schleich and Kästle (2002) reported it from the Chitwan National Park. The specimen was recorded at Ghodemasan area basking on a rock (Fig. 36) in November 2016.

Fig. 36. *Rhabdophis subminiatus. Photograph by Dip Prasad Chaudhary/NTNC-BCC.*

Viperidae (Oppel 1811)

Daboia russelii (Shaw and Nodder 1797): A single individual was observed from Bhata on the way to Rambhori grassland. The individual was basking near a gabion wall (Fig. 37).

Fig. 37. *Daboia russelii. Photograph by Santosh Bhattarai.*

Trimeresurus albolabris (Gray 1842): Two individuals were observed at Kamini Daha in March 2014 and June 2015. The third individual was observed from Ramauli-Pratapur in December 2016 (Fig. 38).

Fig. 38. *Trimeresurus albolabris. Photograph by Kapil Pokharel/NTNC-BCC.*

Testudinidae (Batsch 1788)

Indotestudo elongata (Blyth 1854): An individual was observed at Ghodemasan. Two rescued individuals were kept at Amlekhgunj-Hattisar. Later, they were released inside the park. Local people, especially business people, like to keep turtles and tortoises in captivity believing they are a sign of good luck for their business (Fig. 39).

Fig. 39. *Indotestudo elongata. Photograph by Santosh Bhattarai.*

Crocodylidae (Cuvier 1806)

Crocodylus palustris (Lesson 1831): An individual was kept in an enclosure in Amlekhjung-Hattisar. Later, it was released in a wetland inside the park at Bhata.

Discussion

Our short expeditions resulted in 22 new species records for the PNP, including three species of frog, two geckos, one Agamid, two skink species, 13 snake species, and one crocodile. The details of new species recorded for the PNP are in Table 1.

The record of *Traschischium tenuiceps* by Kästle et al. (2013) from the PNP needs to be verified as the elevational range of the species in Nepal is 1,500–2,400 m (Schleich and Kästle 2002). We presume that the species was mistakenly reported from the PNP.

Our survey mainly focused on daytime searches due to logistics. It is highly likely that many other amphibians and reptiles remain to be added to the list, especially fossorial and arboreal species. During our survey we failed to document *Eryx johnii* (Russell 1801) as this species is frequently observed in nearby areas.

Among the species we recorded, *Varanus flavescens* and *Python* sp. are legally protected species in Nepal. The pythons are the only legally protected snake species of Nepal which has been accorded the highest degree of protection under the National Parks and Wildlife Conservation Act, 1973. The Act has included the python in the Schedule-I as *Python molurus*. In 2009 *Python bivittatus* was elevated to specific status, and the occurrence of *Python molurus* in Nepal is doubtful (Bhattarai 2014). Therefore, we suggest *P. bivittatus* be listed in the Act instead of *P. molurus*.

The IUCN has evaluated the tortoise *Indotestudo elongata* as an endangered species. Similarly, *Crocodylus palustris*, *Ophiophagus hannah*, and *Python bivittatus* have been categorized as vulnerable species. The rampant killing of snake species in the buffer zone of the PNP is an observed threat. Buffer communities perceive all snakes to be venomous despite the fact that only 17% of Nepalese snakes are venomous (Bhattarai et al. 2017; Sharma et al. 2013).

The national east-west highway bisects the park in the Amlekhganj-Pathlaiya section where many wild species are frequently observed trampled by the vehicular movement. The regular monitoring of this section will reveal the extent of wildlife loss due to vehicles.

The PNP shares its western boundary with Chitwan National Park, and the Siwalik hill in the North might have unique species as this park has comparatively drier habitats. We believe detailed inventory will further increase the species richness and diversity of the park.

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Range extension of *Cyrtopodion himalayanus* Duda and Sahi, 1978 (Reptilia: Sauria) in Jammu Province of State Jammu and Kashmir from District Doda, Northern India

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***Abstract.*—Documented are new distributional records of the poorly-known Gekkonidae *Cyrtopodion himalayanus* from the Doda region of Jammu and Kashmir State (India) based on specimens collected in three localities of the Doda region (Village Nai-Bhallara, Village Chagsoo, and Village Zazinda). Presented are notes on the morphology and coloration of the species in Doda, as well as photographs and a map indicating the known localities of *Cyrtopodion himalayanus*. This record represents an extension range of 60–80 km from the earlier reported locality of the species. The species *Cyrtopodion himalayanus* is the sole representative of the group *Cyrtopodion*, documented four decades ago from Kishtwar town of District Kishtwar (formally under District Doda) in the state of Jammu and Kashmir.**

Keywords. Gekkonidae, new distribution, Kishtwar, reptiles, visual encounter survey, morphology

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Introduction

The state of Jammu and Kashmir includes three main areas: Jammu, Kashmir, and Ladakh, which are different from one another in terms of topography, altitude, and climate. District Doda geographically falls in the outer Himalayan ranges and comes under the Jammu province of the state Jammu and Kashmir. District Doda also falls under seismic zone-V as per IS 1893 (Part I): 2002 and situated between 33°08′N, 75°32′E at an average elevation of 1,107 m asl. The studies associated with other faunal components of the state have been increasing over the past years, whereas the study associated with reptilian fauna of the region is very scant. Fenton (1910) was a pioneer in ophidian studies in the state of Jammu and Kashmir. Since the publication of *The Fauna of British India* by Boulenger (1890) and Smith (1935), very little attention has been given to its reptilian fauna. The work of Das et al. (1964), Duda and Koul (1974), Murthy and Sharma (1976), and Murthy et al. (1979) enlisted some records of reptiles, but their studies focused on only two regions of the state (Jammu and Kashmir), Kashmir and Ladakh.

There was no information regarding reptilians from Jammu province until Sahi (1979), who has conducted an extensive survey of Jammu and Kashmir state for the herptiles and reported 76 species. He stated that the Jammu province of the state is the richest of the two regions of the state in terms of reptilian diversity. The distinguishing oversight of references to the Doda region of Jammu province (Jammu and Kashmir state) on herptiles of the state undoubtedly indicates the lack of any faunistic survey ever having been conducted in this part of the state since Sahi (1979).

Methodology

We have conducted surveys during the years 2014–2015 following the visual encounter surveying method (Campbell and Christman 1982). The survey was conducted from March to mid-June for both years (2014 and 2015). We have photographed the specimens using a digital camera (Sony HX300), and geo-coordinates were recorded using GPS (GPS test). Morphological measurements of the specimens collected were recorded by using a digital caliper (Precision 150). The specimens sighted

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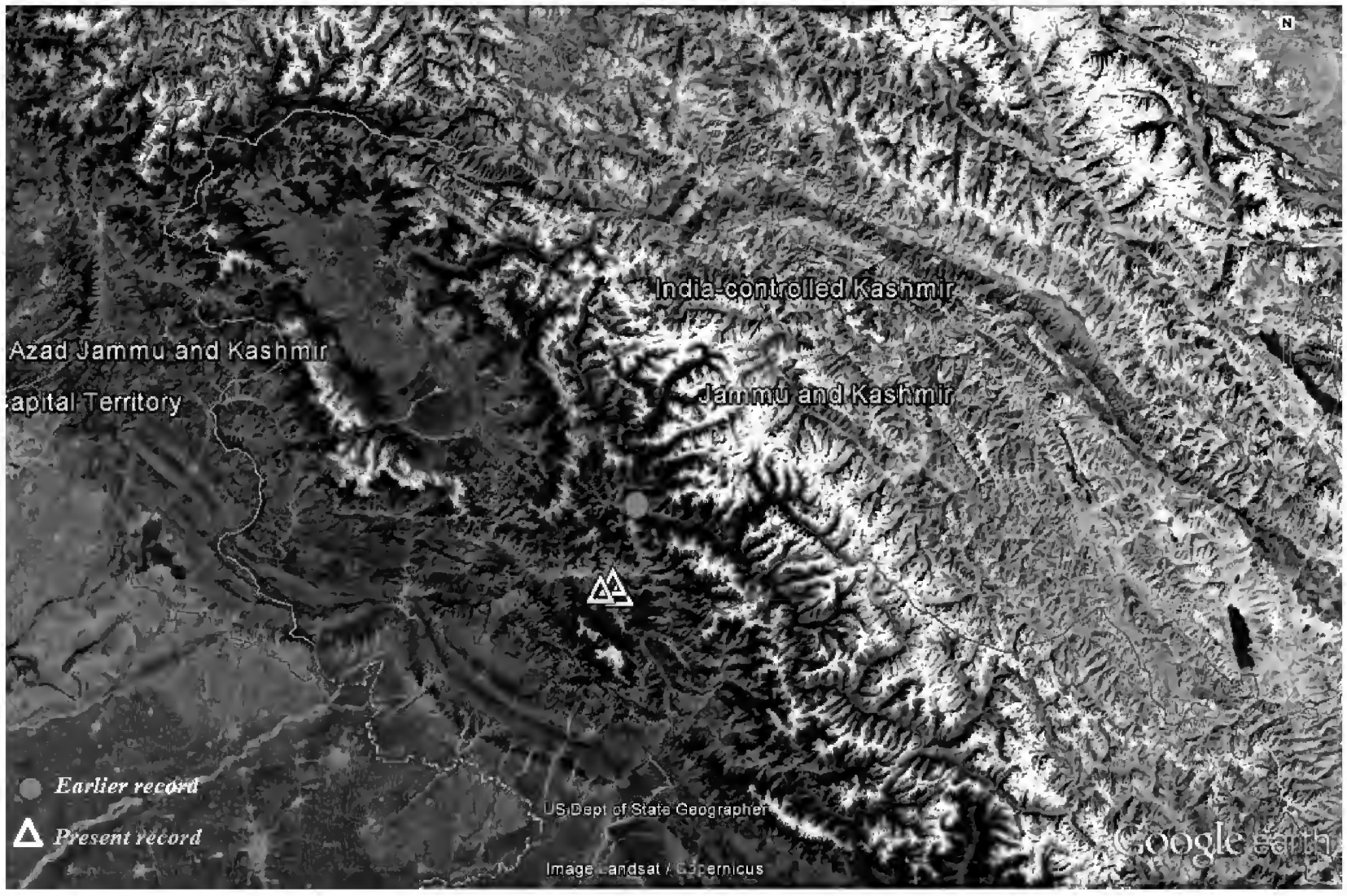

Fig. 1. Depicting the localities of distribution (Earlier and Present).

were identified with the help of descriptions and keys given by Sahi (1978).

Results and Discussion

Cyrtopodion is a complex group of Asian geckos comprising of 37 species at present (Uetz and Hošek 2015). While surveying the herptiles of the state (Jammu and Kashmir), Duda and Sahi (1978) had collected eight specimens of *Cyrtopodion himalayanus* (75.7E, 3.3N; 1,700 m) at an elevation of 1,700 m from a house in Kishtwar town on 8 May and 24 October, 1978 in District Kishtwar (formerly under District Doda) and described it as a new species of *Cyrtopodion* (earlier = *Cyrtodactylus* (Duda and Sahi 1978)) (Fig. 2). During an investigation (2014–2015) of reptilian fauna in District Doda, we sighted individuals of the species from three localities different from the earlier record (Fig. 1). The stations are Nai-Bhallara (33°05'20.69"N, 75°42'30.24"E; 1,808 m asl), Village Chagsoo (33°07'33.27"N, 75°40'11.50"E; 1,743 m asl), and Village Zazinda (33°5'34.48"N, 75°38'19.74"E; 2,157 m asl). The stations Nai-Bhallara and Village Chagsoo fall under Tehsil Thathri of District Doda, whereas the latter falls under Tehsil Bhaderwah. The stations are about 60–80 km away from the Kishtwar town. The specimens were sighted near human settlements (inhabited debris and house wall crevices). The specimens were studied alive and released at the same place after ensuring their morphological and physiological characteristics.

The morphological and physiological characteristics of *Cyrtopodion himalayanus* sighted during the present investigation are given in Table 1. Various distinguishing features of every individual were observed, such as: greyish body with dark brown reticulation; brown head with a distinct streak from nape to snout passing through eye on each side; inverted snout; small nostrils placed dorsolaterally; ten upper-labials; eight lower-labials; snout longer than the distance between the eye and ear opening; ear opening sub-oval; clawed digits; claws embedded between two large shields.

Duda and Sahi (1978) analyzed and documented the body length of the specimens to be between 125 mm and 140 mm during their study, whereas morphological characteristics of *Cyrtopodion himalayanus* of the current study reveal specimens with lengths from 115 mm to 136 mm.

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Fig. 2. *Cyrtopodion himalayanus* **(A)** Enlarged lateral view of head. **(B)** Full lateral view of the body.

Table 1. Variations in various characteristics of specimens of species *Cyrtopodion himalayanum*.

Characteristics	Range
Full body length	115 mm–136 mm
Snout-vent length	61.89 mm–68.79 mm
Tail length	53.11 mm–67.21 mm
Head width	11.75 mm–14.91 mm
Head length	16.35 mm–21.38 mm
Snout to mouth length	12.13 mm–14.58 mm
Intra-orbital distance	1.86 mm–3.10 mm
Eye diameter	5.22 mm–5.45 mm
Nostril to eye length	5.35 mm–6.16 mm
Ear diameter	1.89 mm–2.80 mm
Nostril to ear length	13.86 mm–15.79 mm
Forearm length	17.80 mm–20.81 mm
Hind arm length	14.97 mm–17.87 mm
Supralabial scales	10/10
Infralabial scales	8/8

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